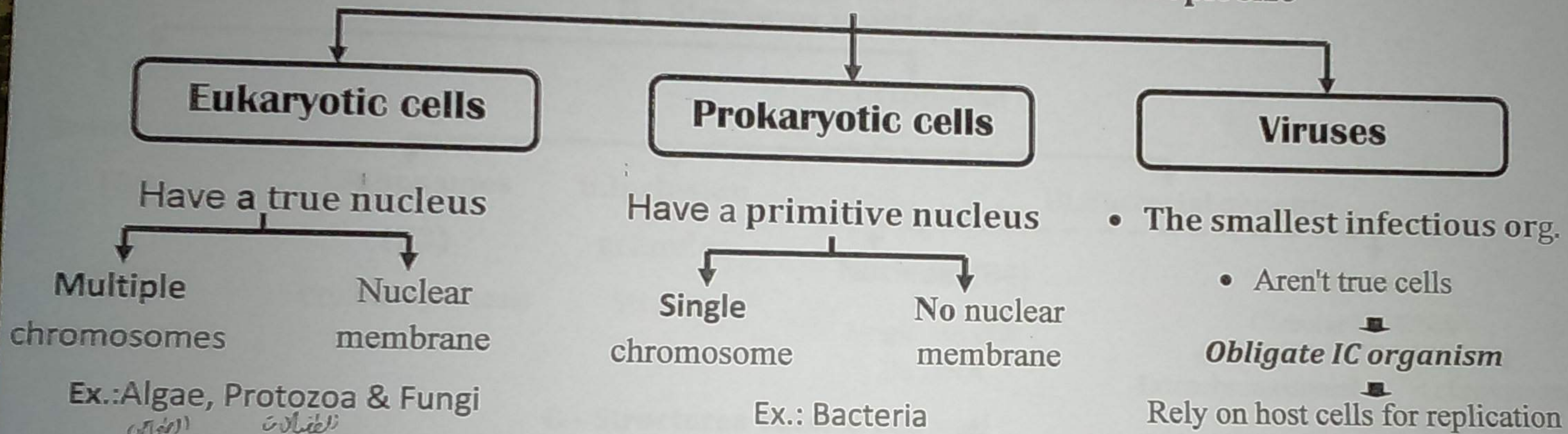


# Important definitions

Microbiology is the study of living organisms of microscopic size



## Items of general bacteriology

1-STRUCTURE OF BACTERIAL CELLS.

2-ANTIMICROBIALS.

3-BACTERIAL GENETICS.

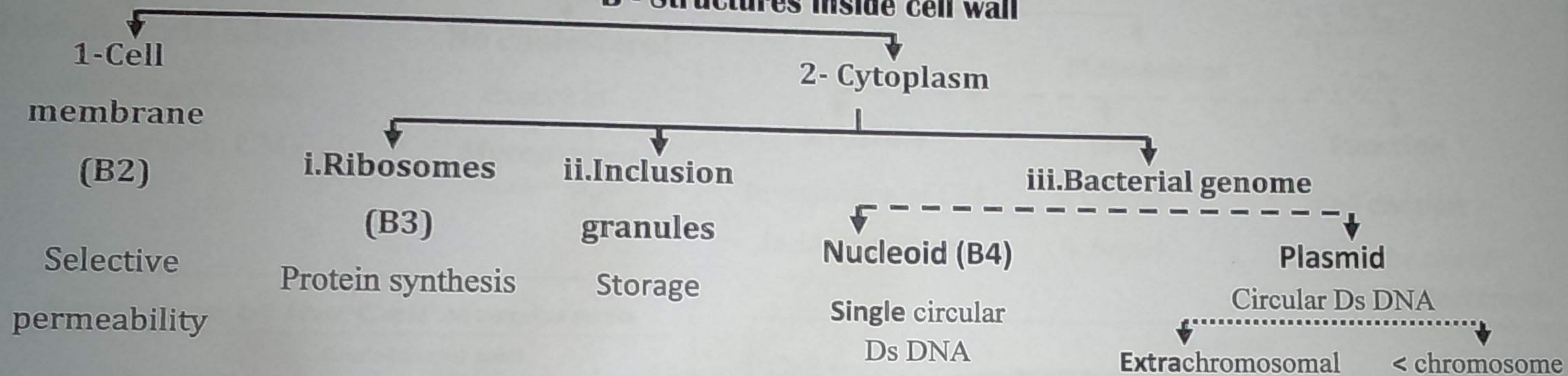
4- HOST MICROBE RELATIONSHIP.

5-BACTERIAL GROWTH & CLASSIFICATION

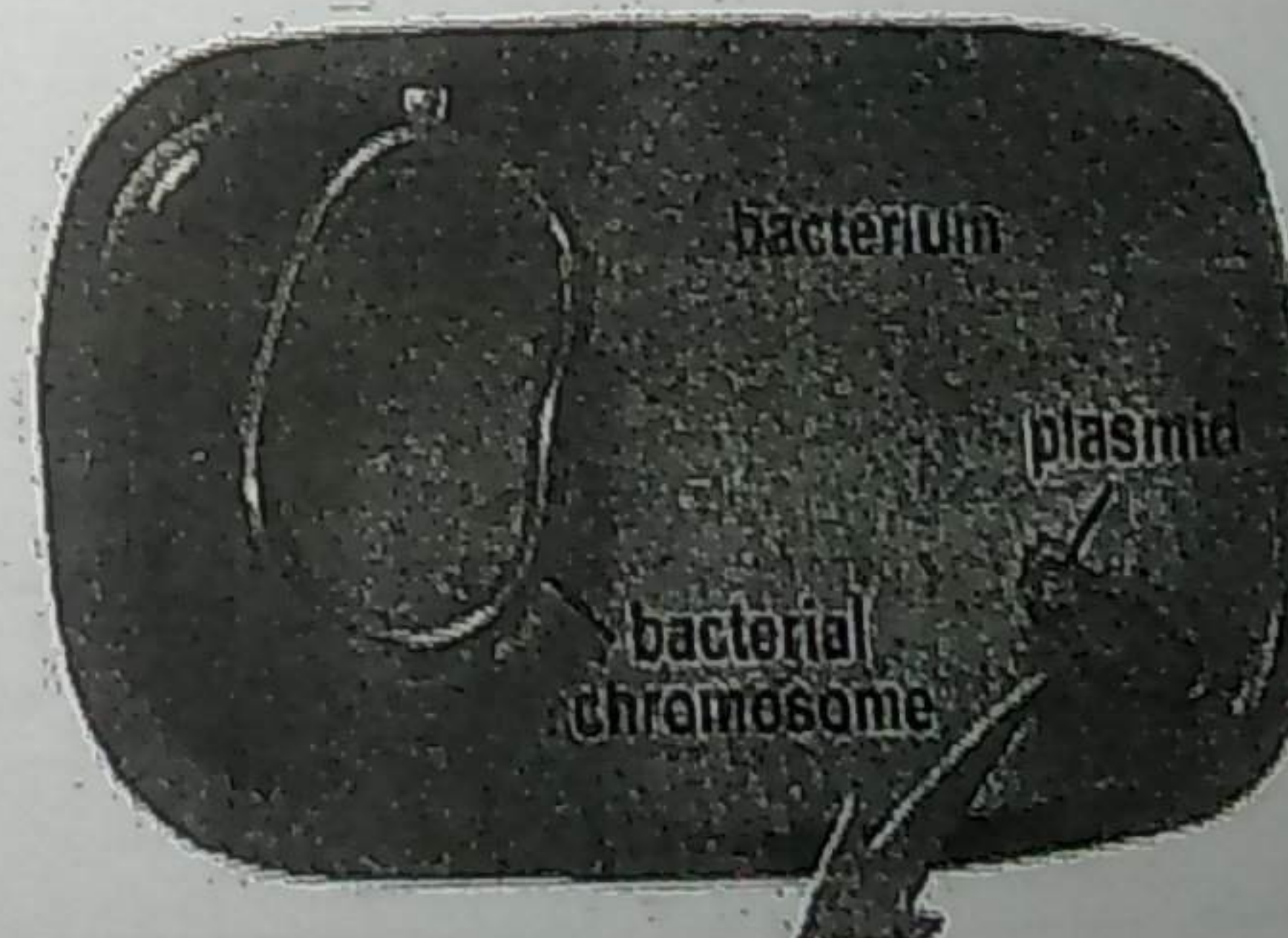
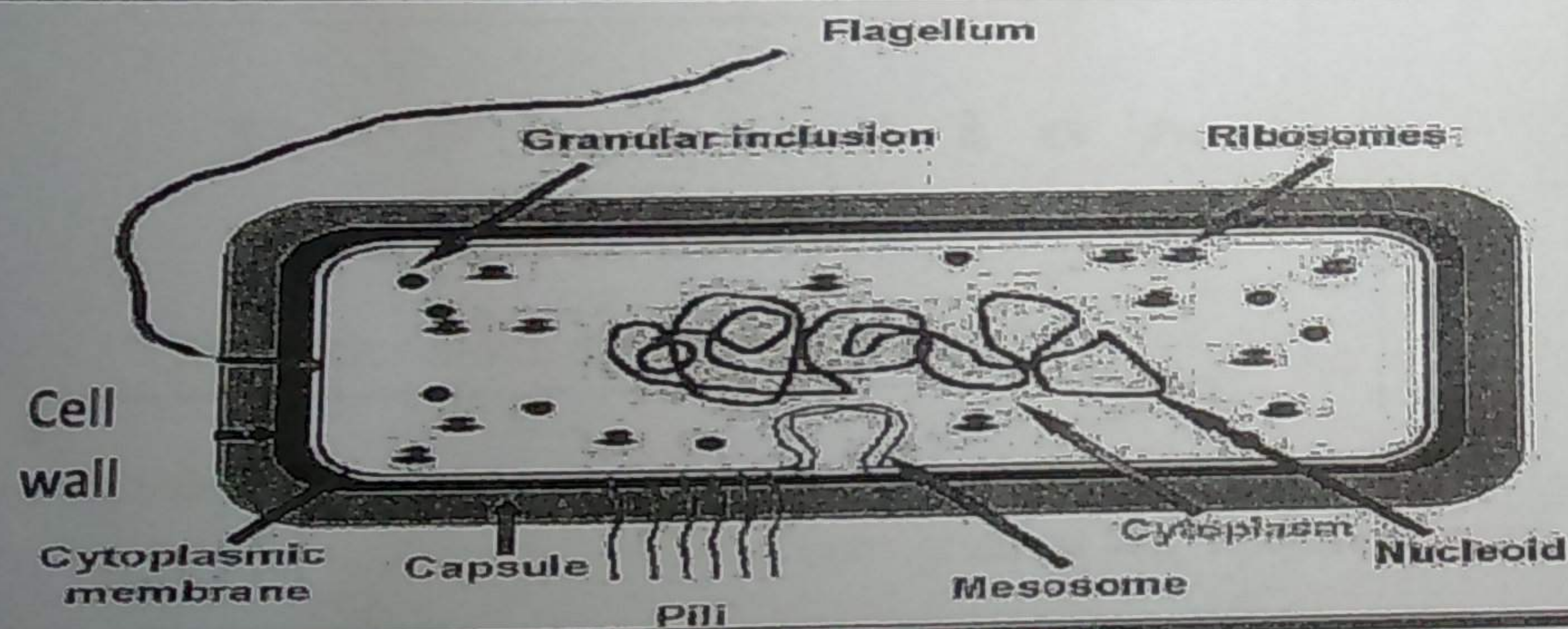
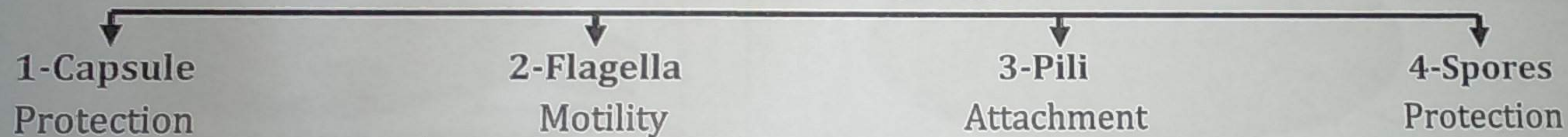
# Bacterial Cell Structure

A - Cell wall ( B1) : Protection

B - Structures inside cell wall



C - Structures outside cell wall



# Cytoplasmic membrane (CM)

## A - Structure

Phospholipid bilayer  
containing proteins  
(as eukaryotic CM)

No cholesterol  
except in  
*Mycoplasma*  
المشربك العنكاف

Mesosomes

Structure

Invagination of CM  
inside cytoplasm

Types

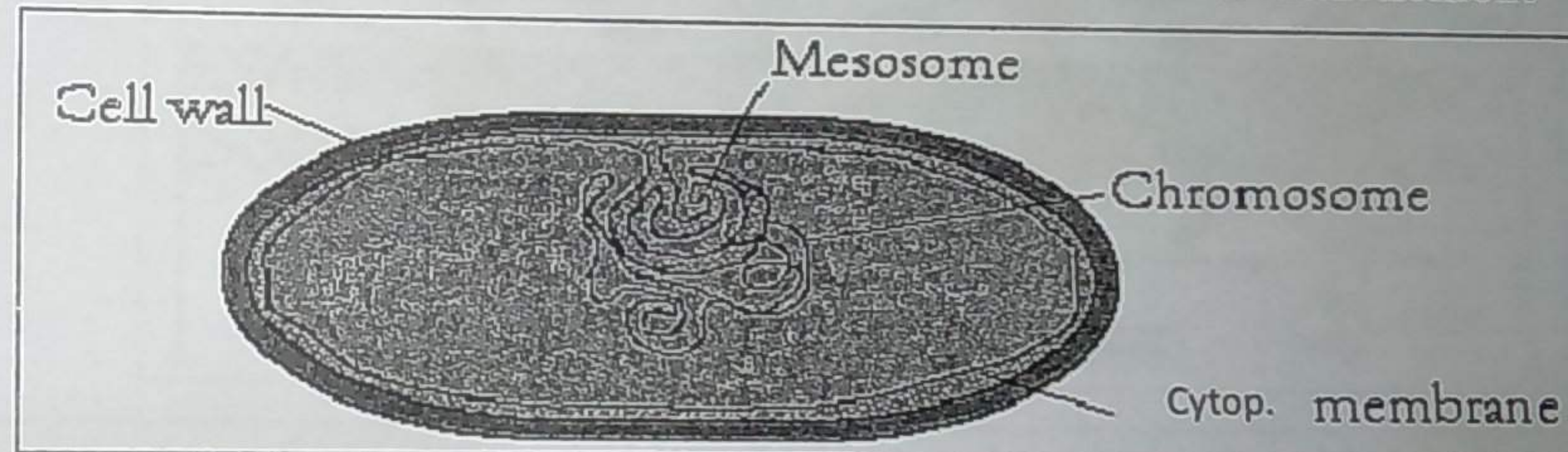
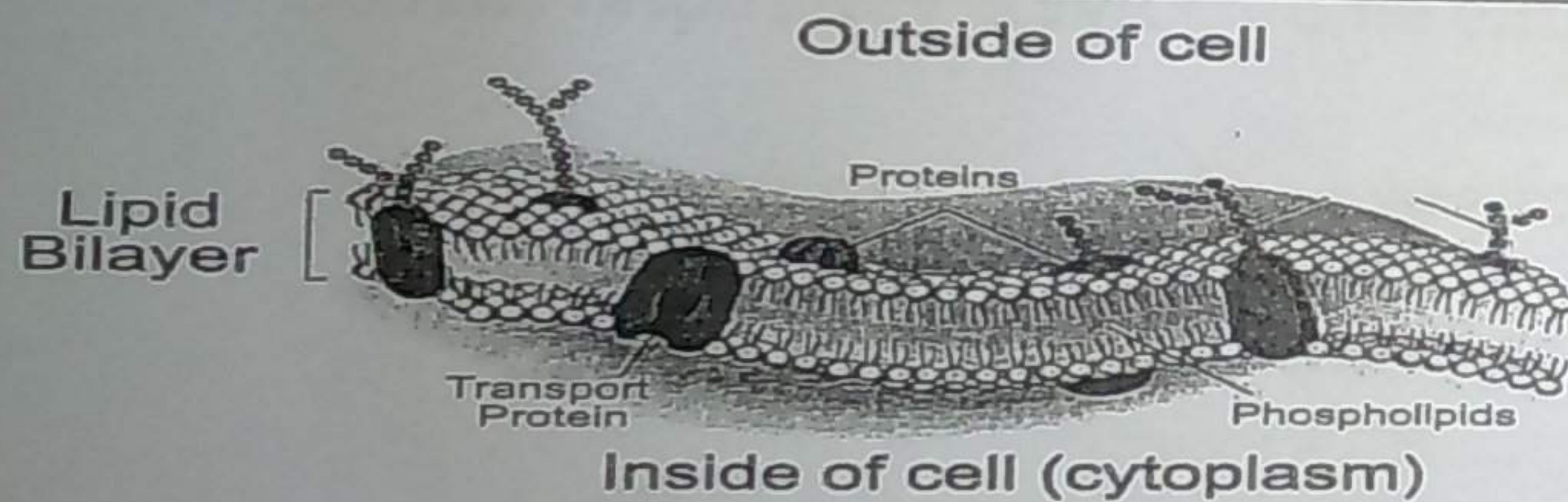
- i. Lateral
- ii. Septal

Function

Cell division

SM are the site of  
chromosomal attachment

### Structure of the Cell Membrane



## B - Functions

### 1 - Selective transport

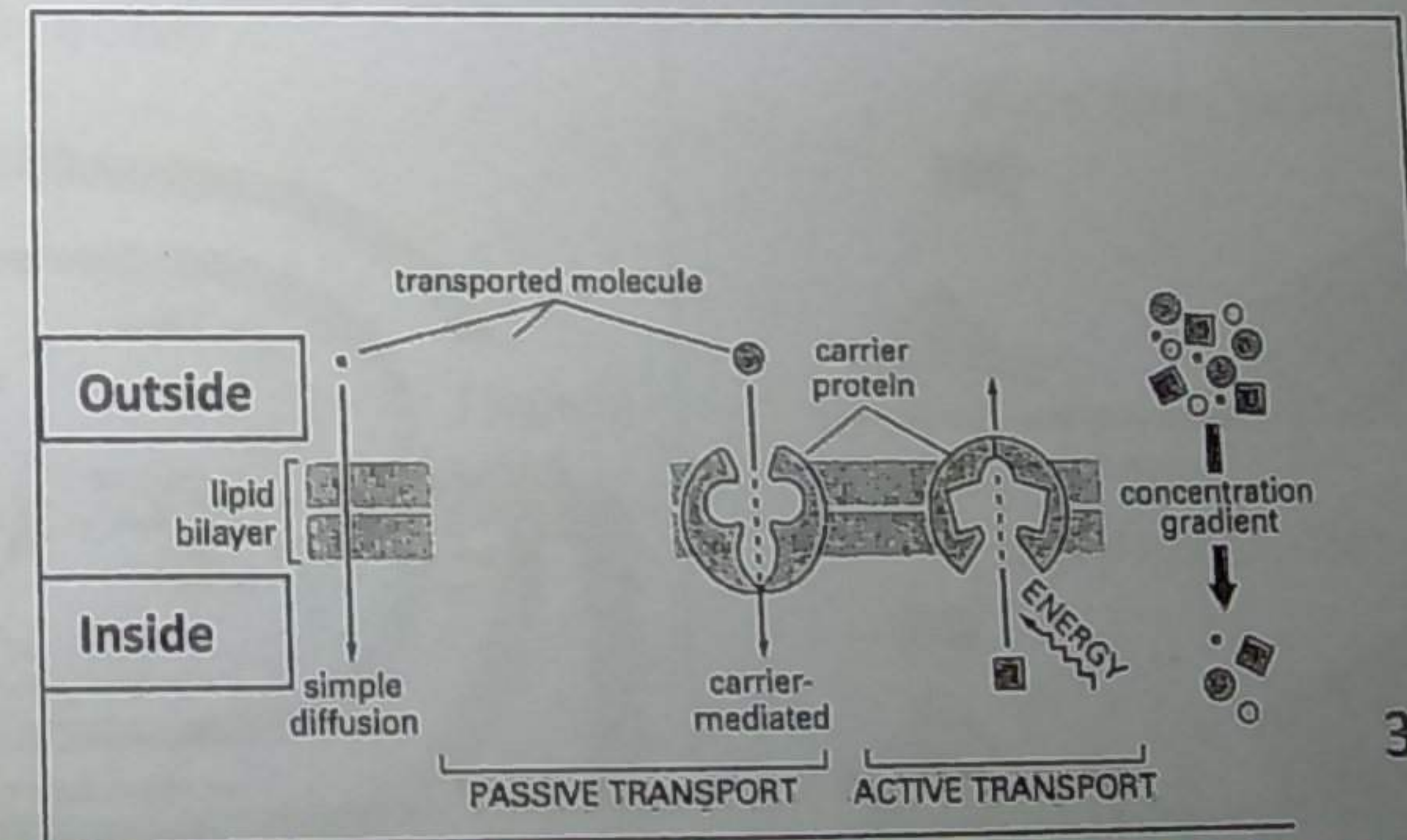
SERC

Molecules move across the membrane by

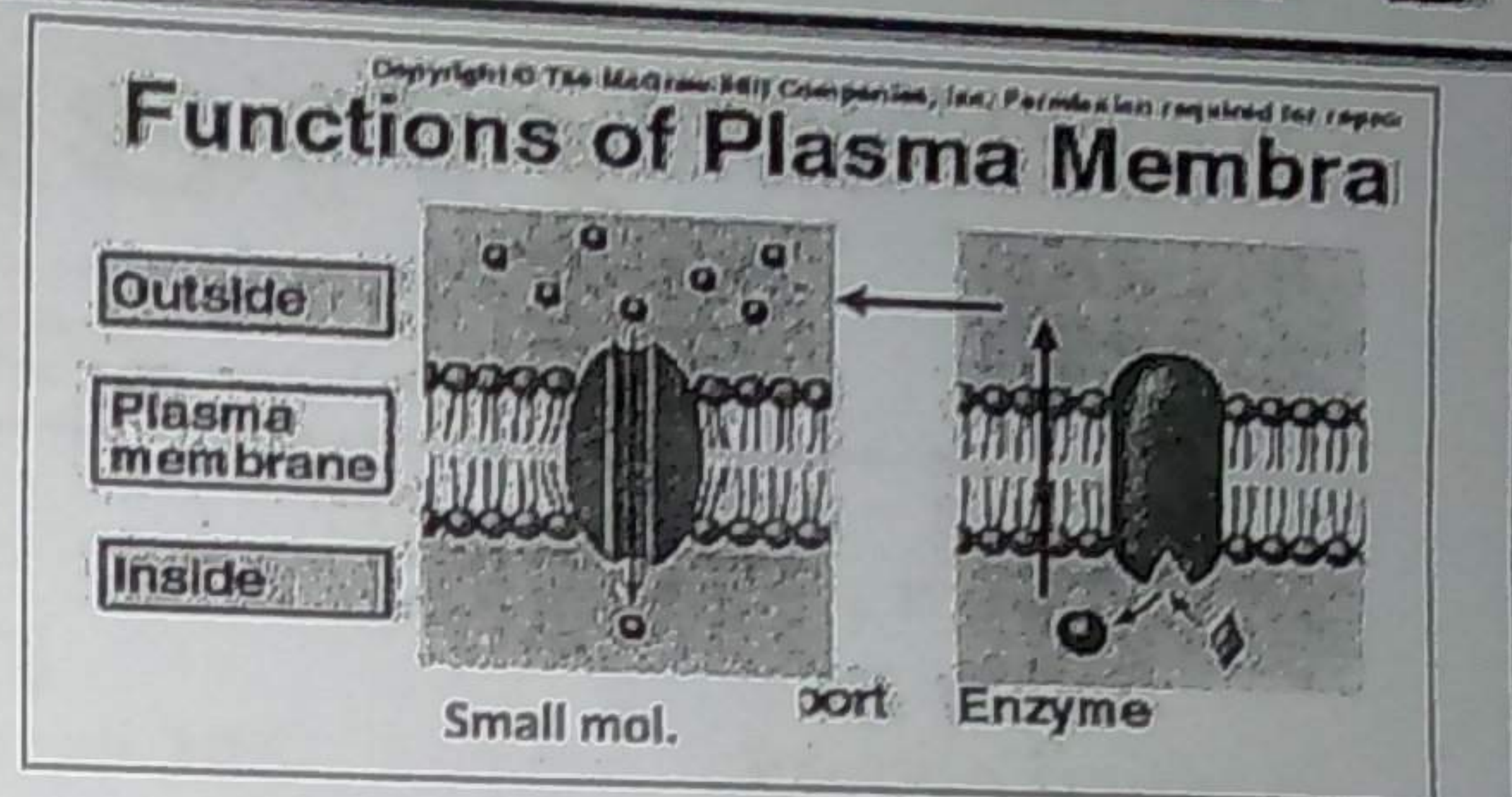
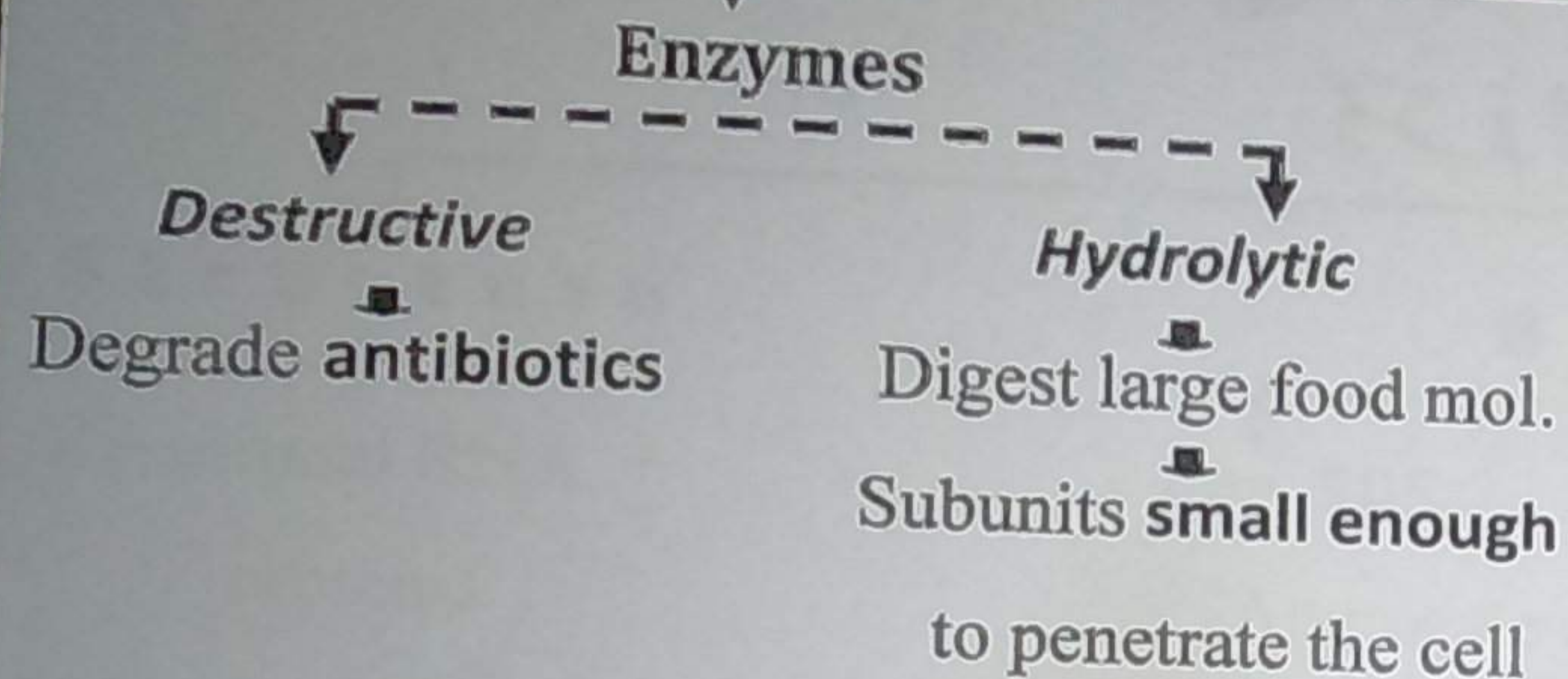
Simple diffusion

Active transport

Against conc.gradient → Requires energy

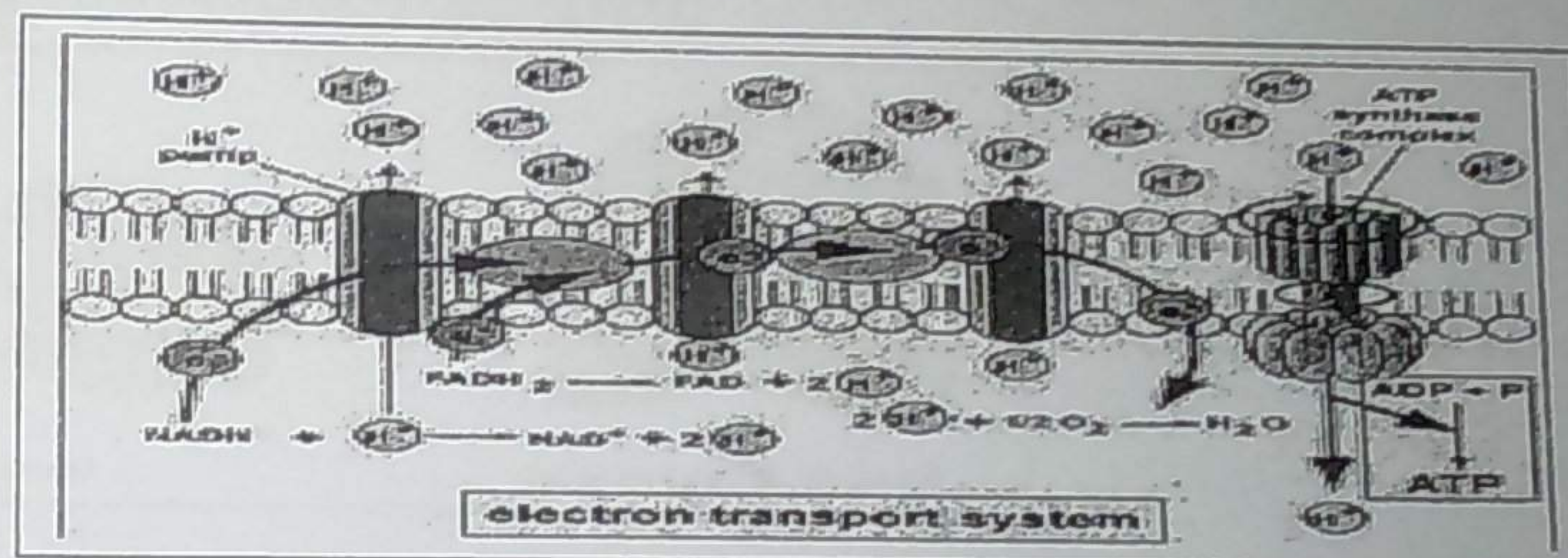


## 2- Excretion of extracellular



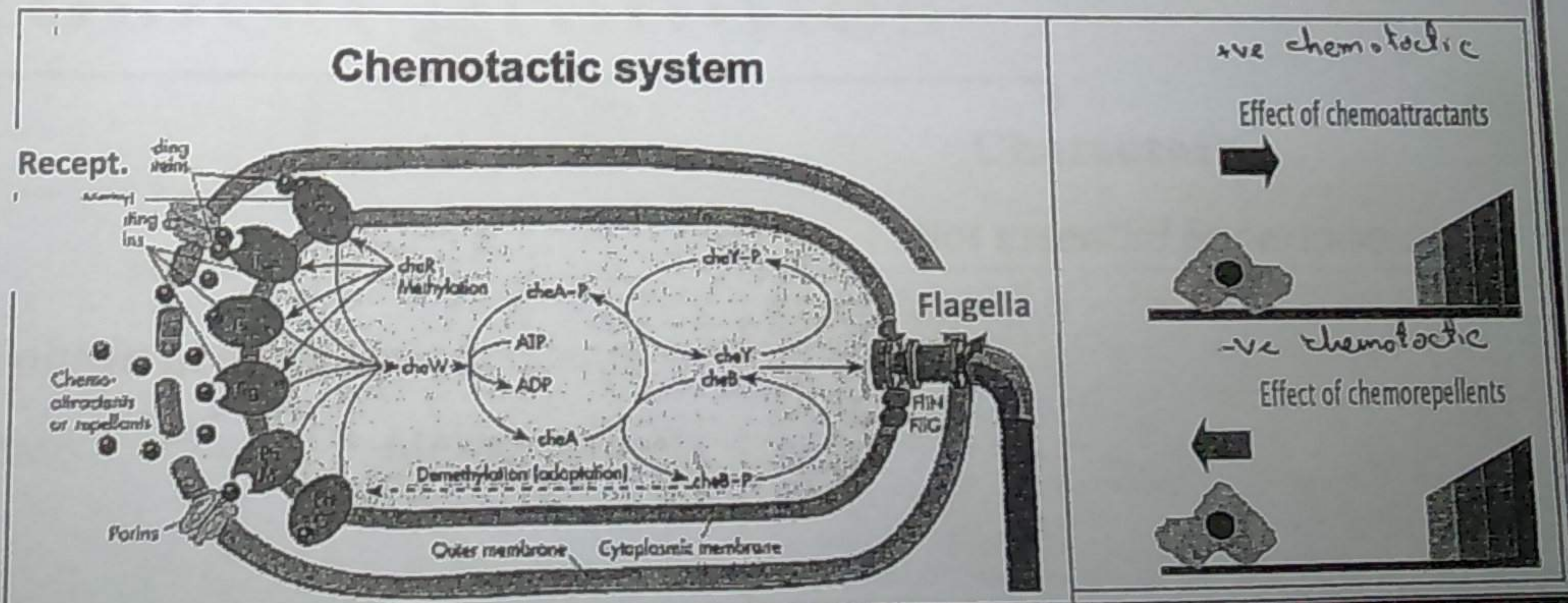
## 3 - Respiration & generation of ATP

Contain respiratory & cytochrome enzymes  
(as *mitochondrial* membrane in eukaryotes)



## 4 - Chemotactic systems

Expresses specific receptors  
↓  
Bind attractants & repellants  
↓  
Send signals to cell interior



# Cytoplasm

## Ribosomes

### Structure

Ribosomal RNA +  
proteins

### Subunits

Large 50S

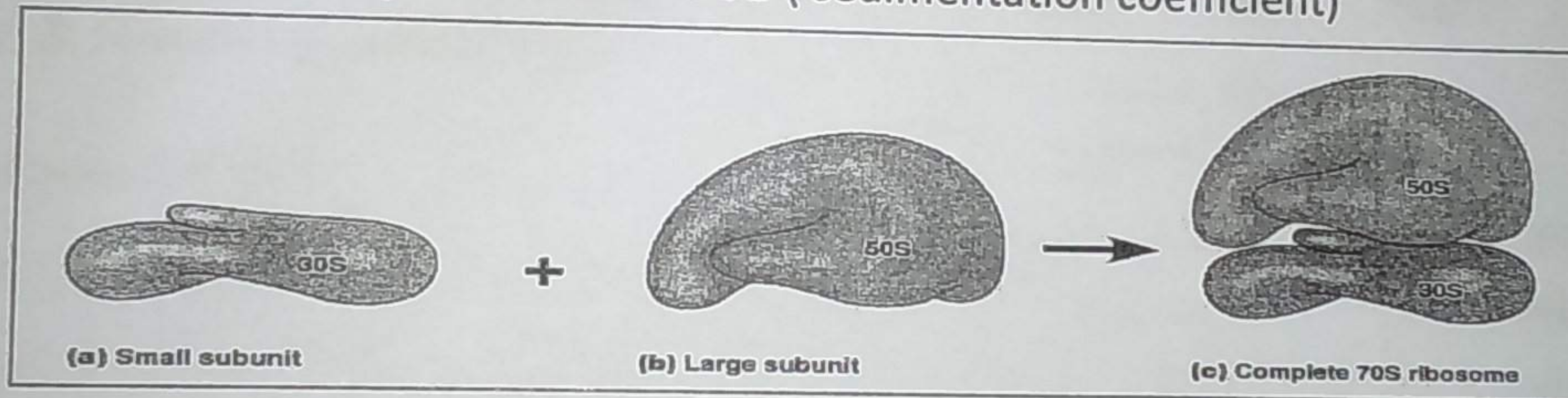
Small 30S

Aggregate during protein synthesis

polyribosome: 70S (sedimentation coefficient)

### Functions

Protein synthesis



## Inclusion granules

### Types

Nutrient reserve  
for cell metabolism

Stored energy

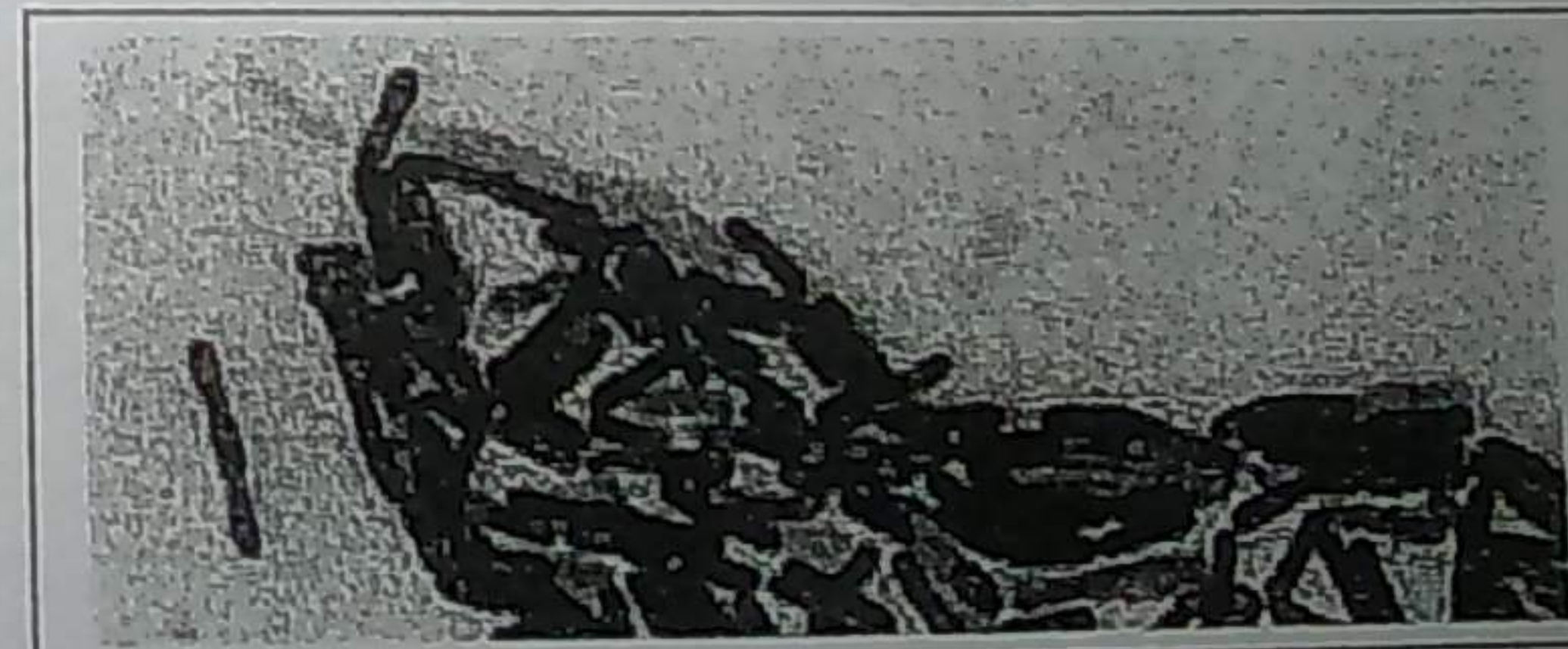
e.g *Volutin granules* in Diphtheria

Polyphosphate

Metachromatic

### Characters

Not essential or permanent



# C e l l W a l l

## I - S t r u c t u r e (absent in Mycoplasma)

Differs between Gram + ve & Gram -ve bacteria

### A - P e p t i d o g l y c a n

Give Reason cell wall of gram +ve bacteria is stronger than gram -ve bacteria?

#### G+ve bacteria

1. **Thick** : 40 sheets → 50% of CW thickness → **Stronger CW**

2. Each sheet is formed of **alternating**

N-acetyl muramic acid & N-acetyl glucosamine.

3. Sheets are connected by :

i. **4 a.a. (tetrapeptide) side chains** (attached to NAM)

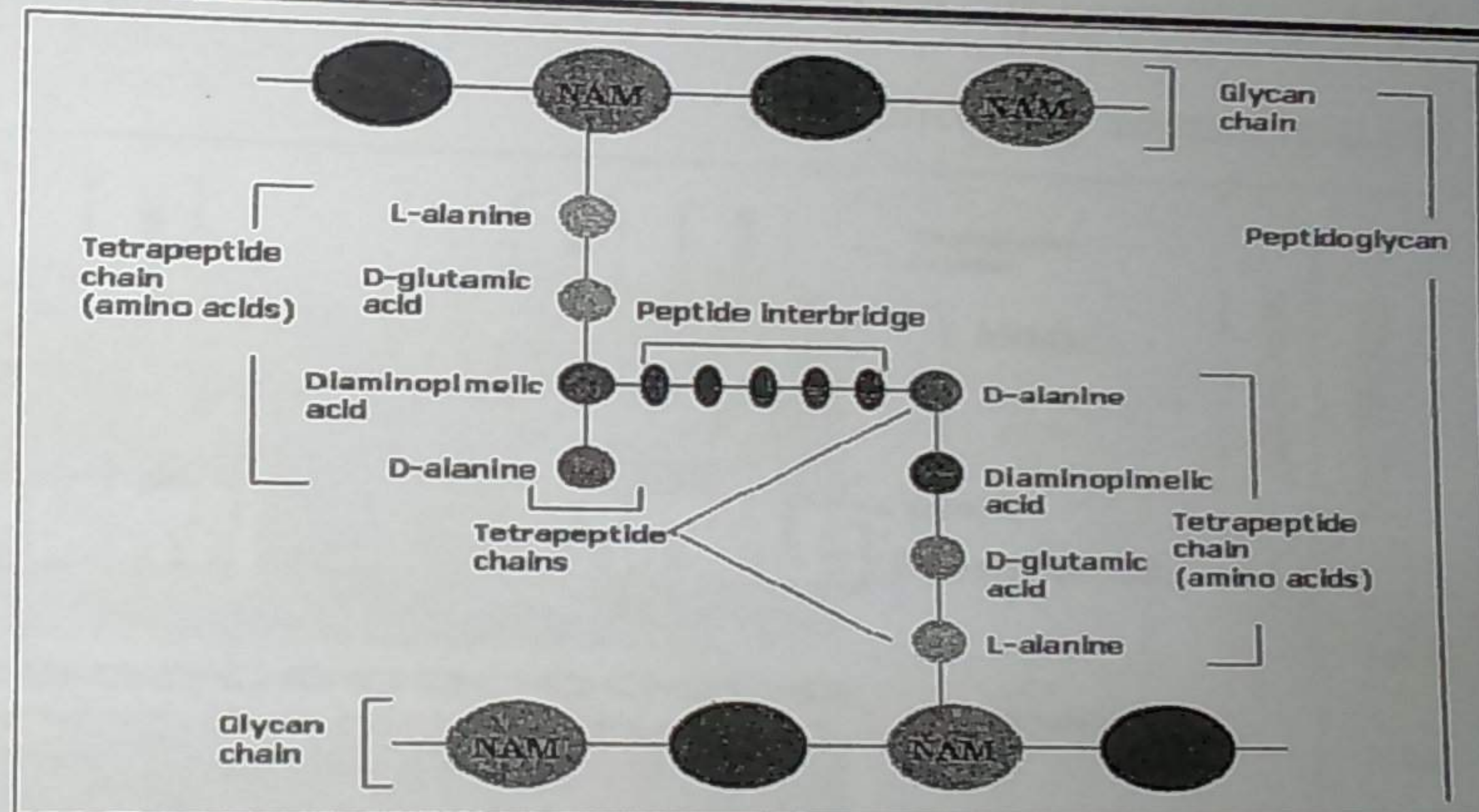
ii. **Identical cross linking peptide bridges**

**F : Rigidity (osmotic barrier) (1)**

Supports weak CM → prevents osmotic rupture

#### G-ve bacteria

1. **Thin** : 1-2 sheets → 5-10% of CW → **Weaker CW**

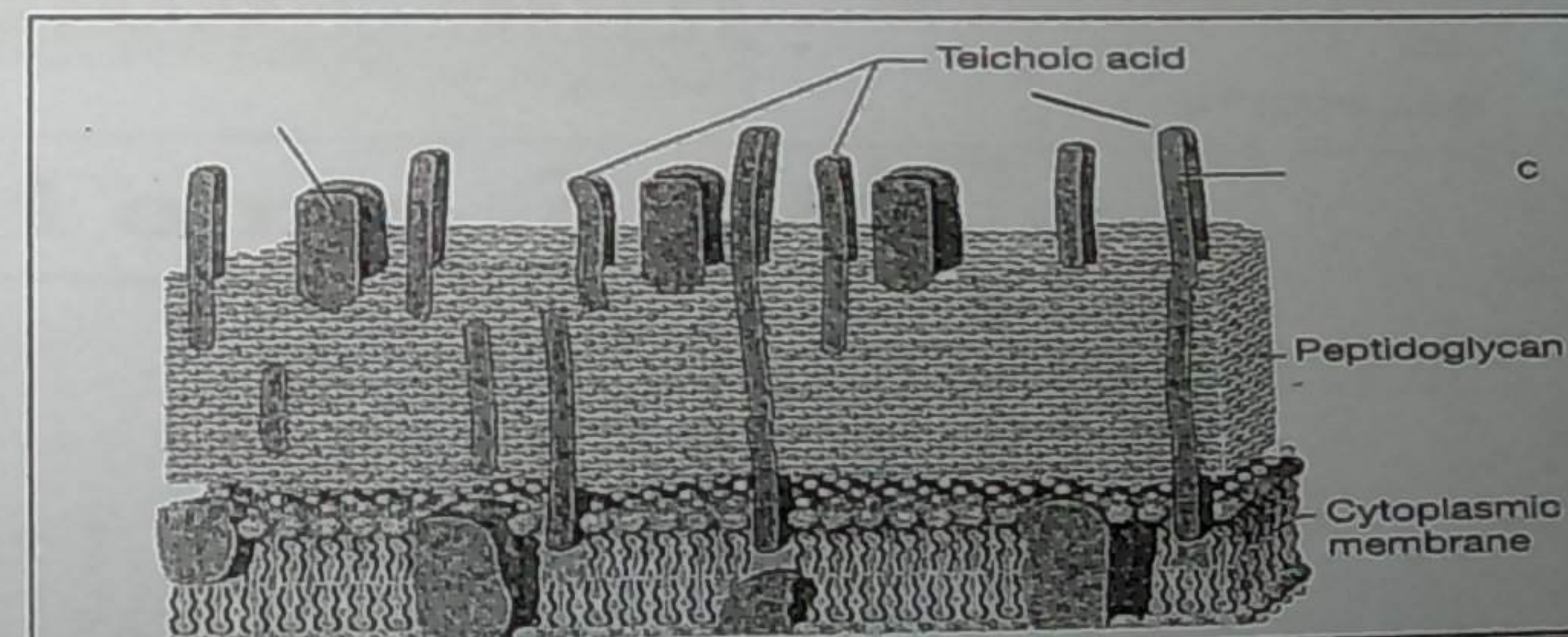


### B - O t h e r l a y e r s o f G + v e b a c t e r i a

**Teichoic acid** (found also in CM)

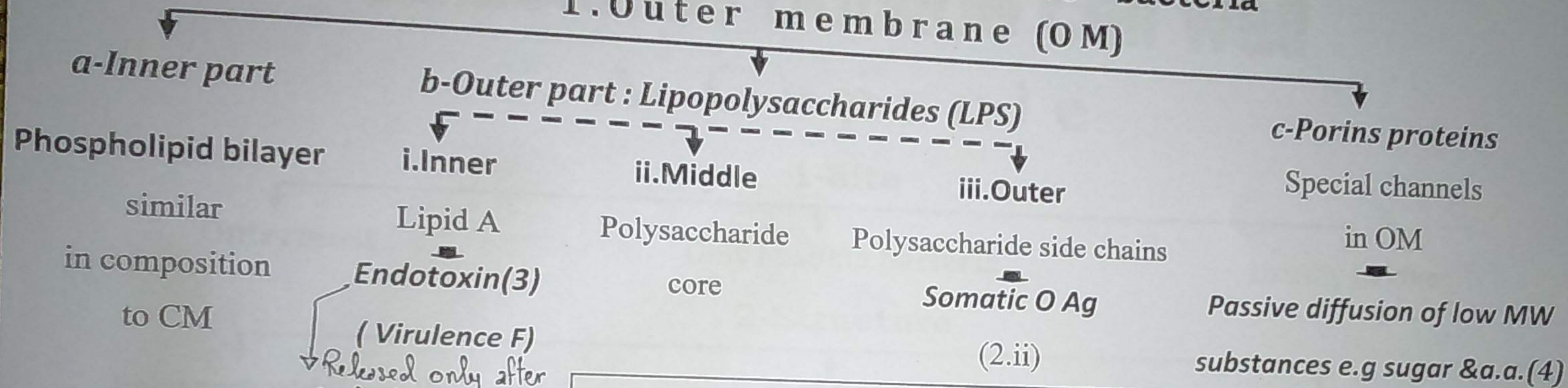
**S** : Glycerol or ribitol phosphate

**F** : Major Ag  
(2.i)



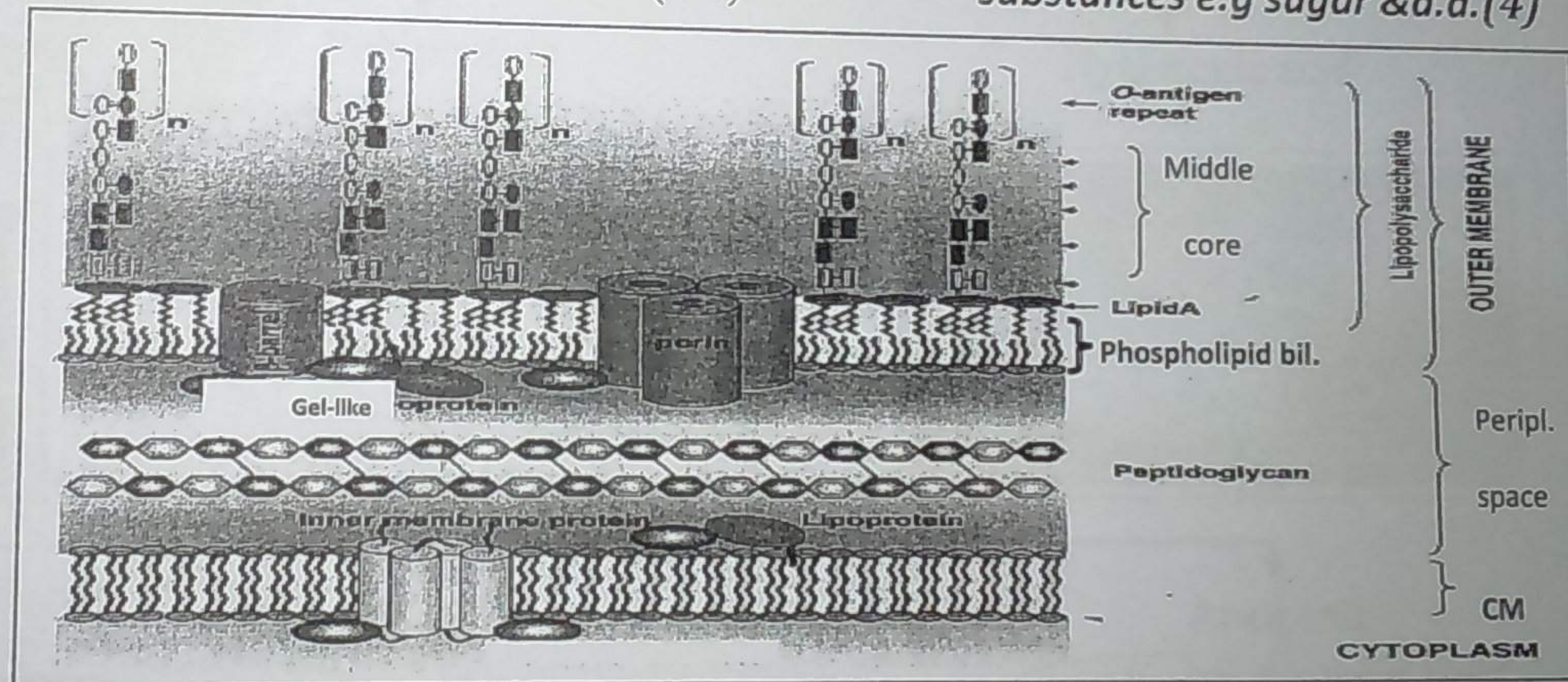
## C - Other layers of G - ve bacteria

### 1. Outer membrane (OM)

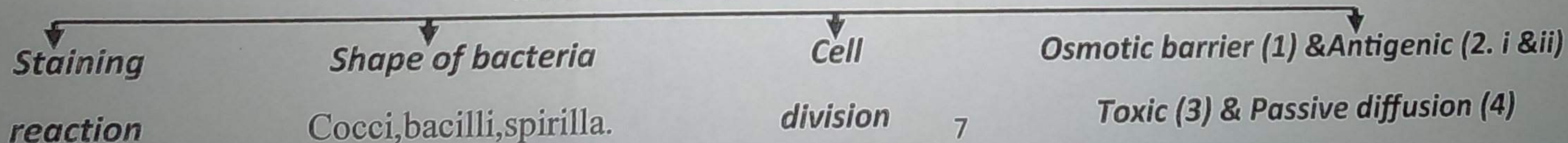


## 2. Periplasmic space

Between CM & OM :  
contain PG + gel-like protein

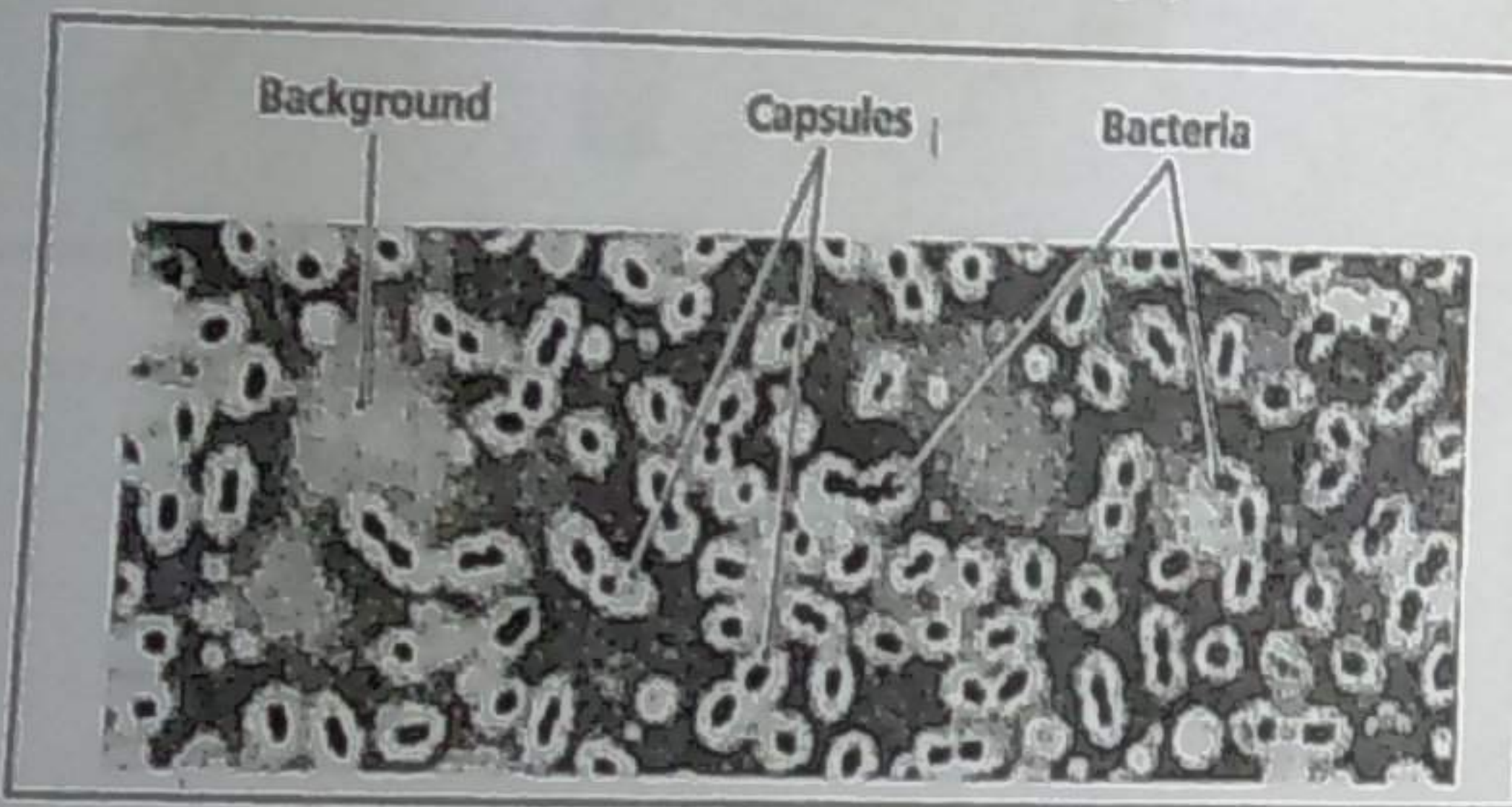
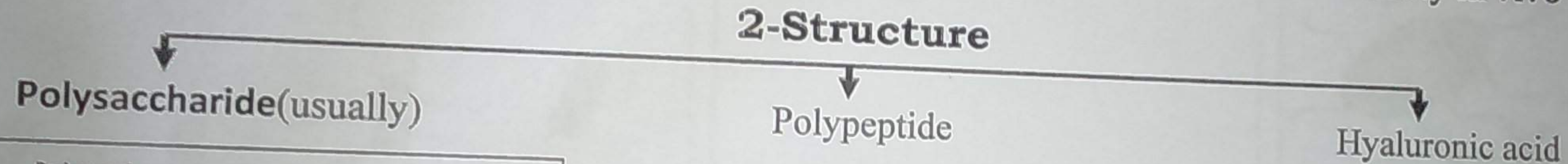
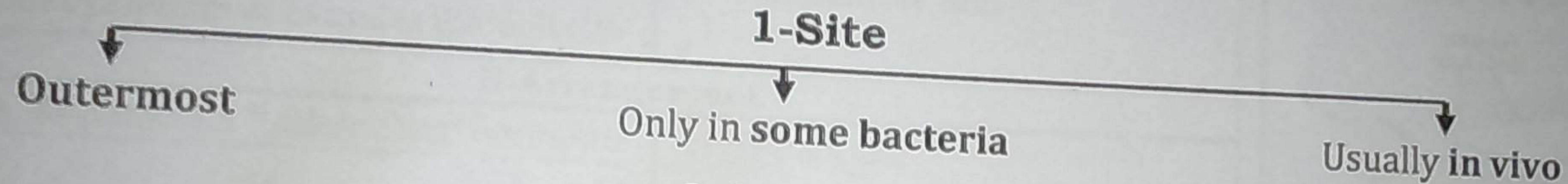


## II - Functions 25 + DOAPT



# Structures outside cell wall

## A- Capsule



**3-Stain**

*Capsular stain* (not stained by Gram)  
?!



### 4 -Functions AAA

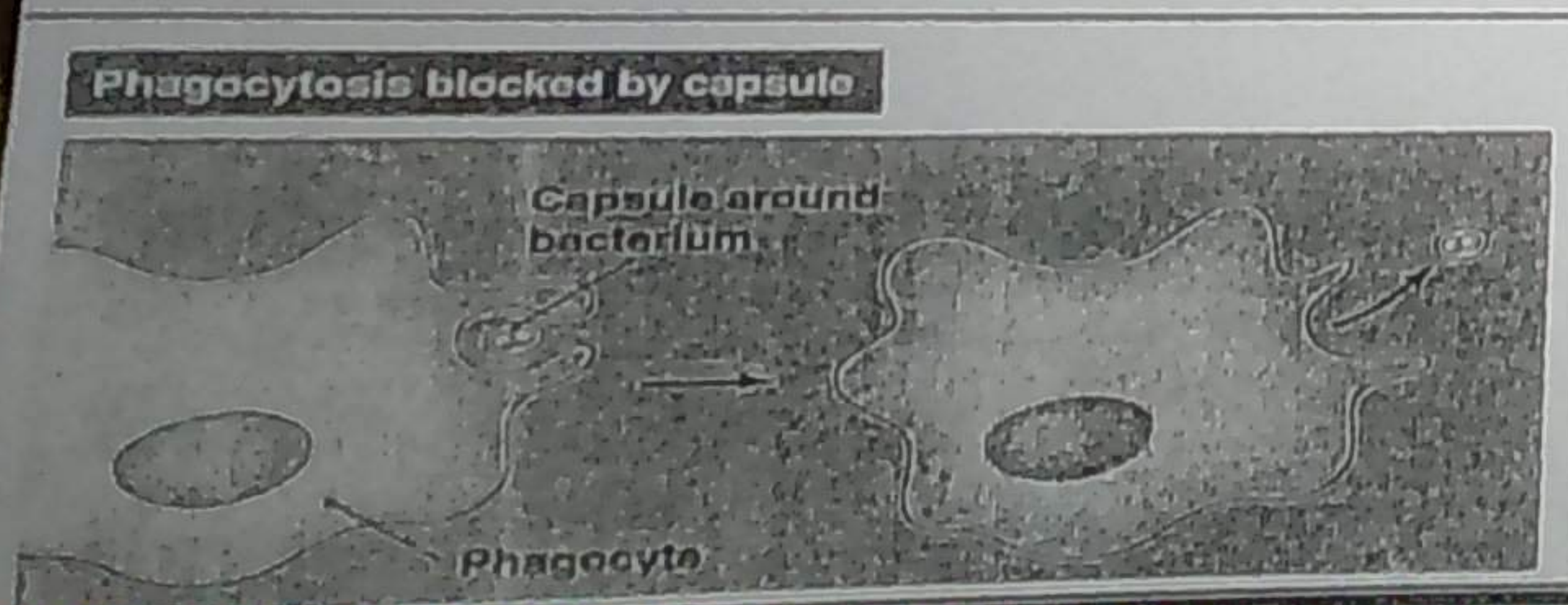
Antiphagocytic → VF

Attachment to mucus membrane

Antigenic

→ stimulate the immune response  
in the body of host

virulence  
[VF]



# B - Appendages

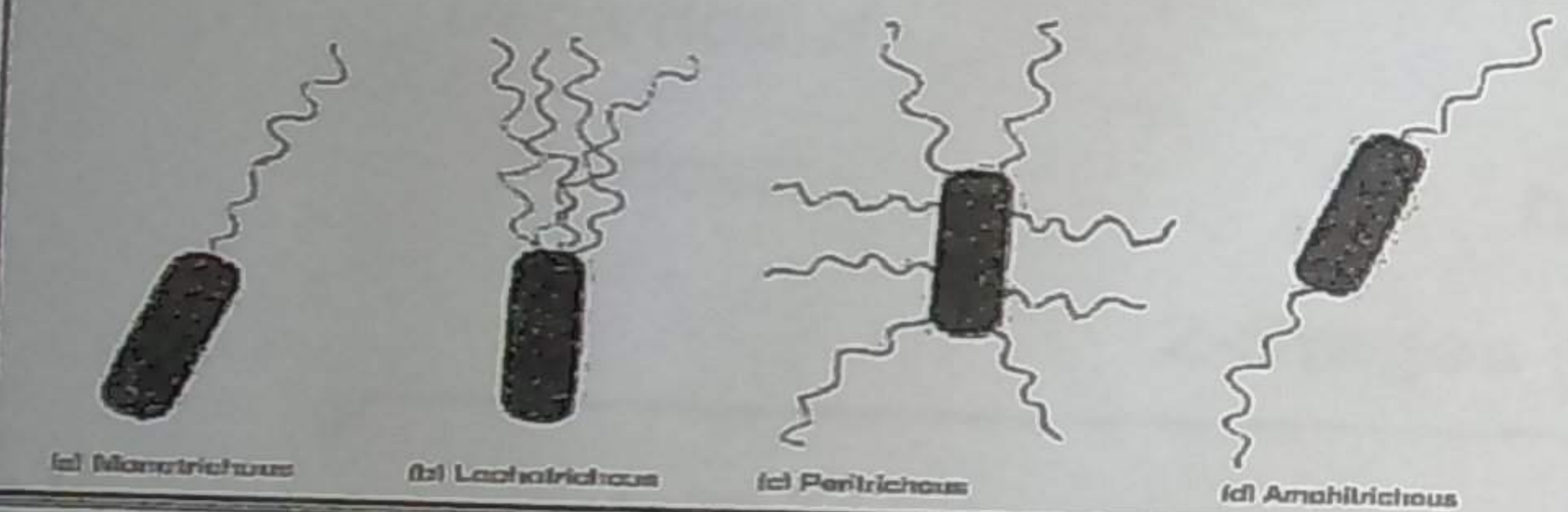
## Flagella

### A-Structure & shape

- i. Formed of a protein cd : **flagellin**
- ii. Long & thick
- iii. Arise from cytoplasm & extrudes through CW

### B-Arrangement

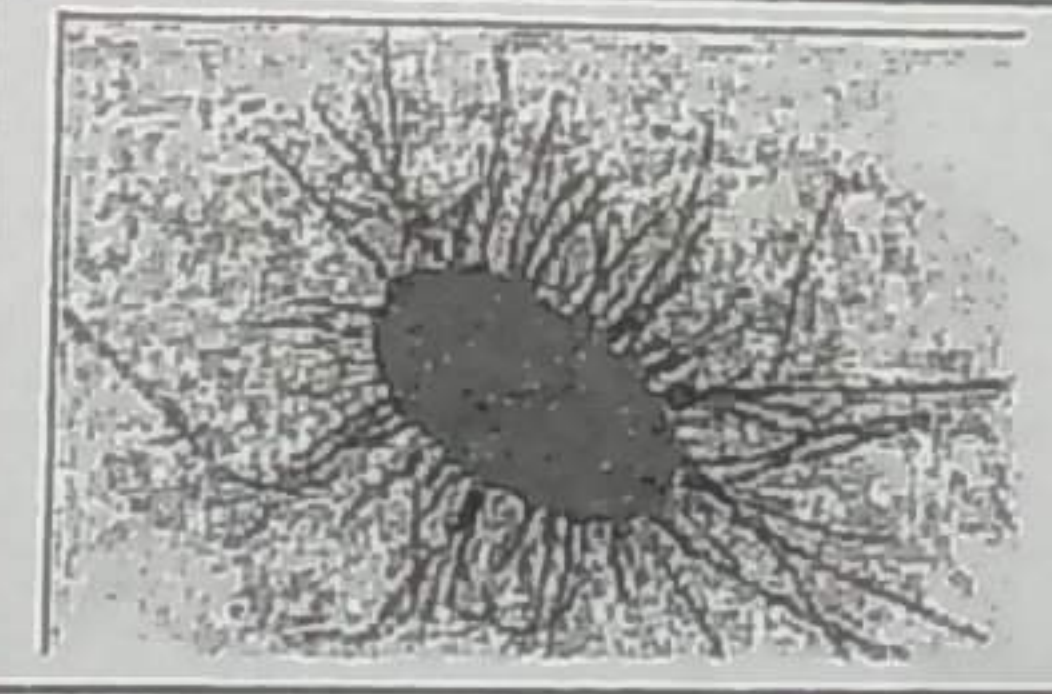
\*Monotrichate \*Amphitrichate \*Lophotrichate \*Peritrichate



### C-Types & functions

1-Motility

2-Antigenic : H Ag



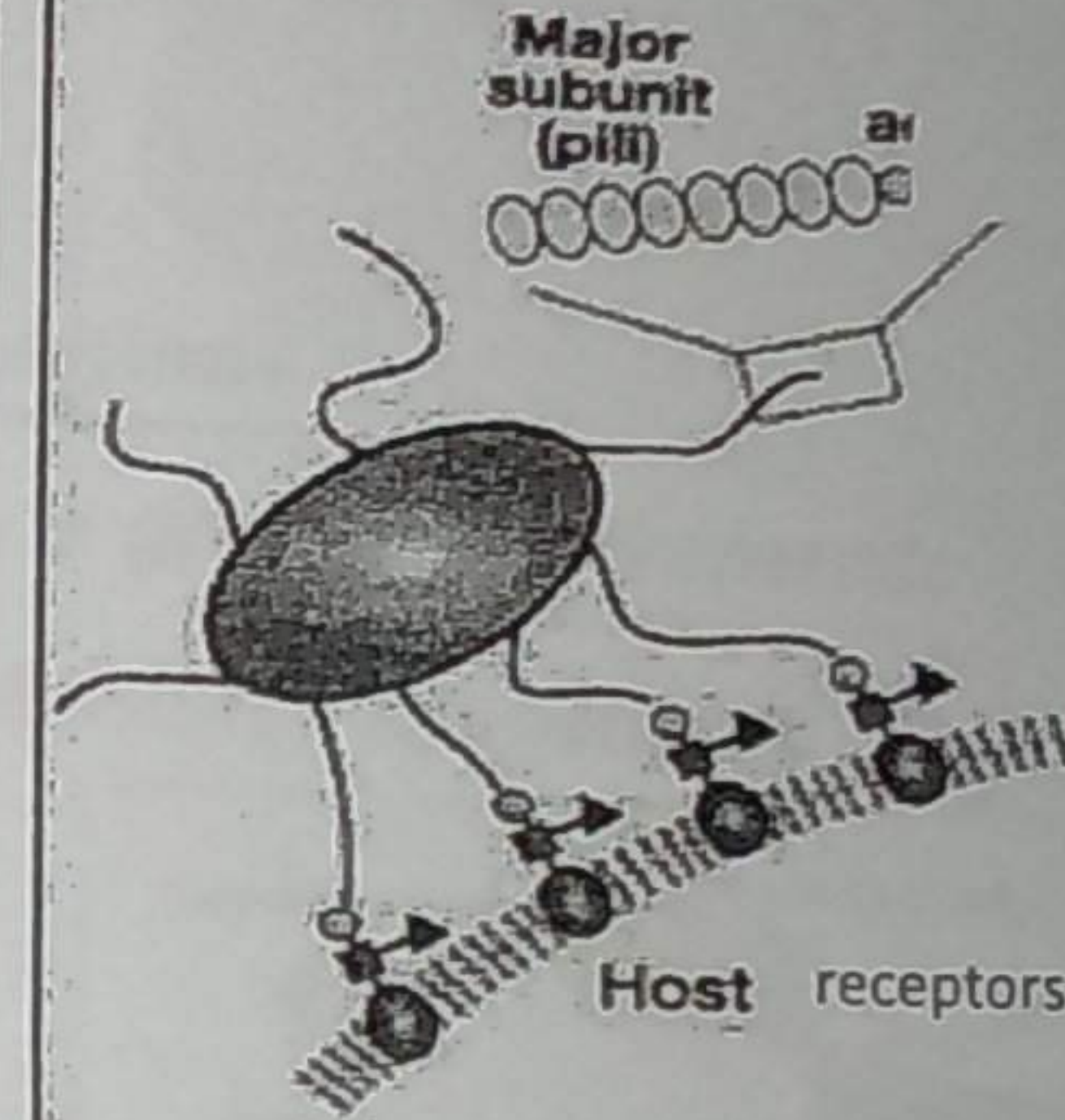
### D-Stain

? Flagellar stain (not stained by Gram)

## Fimbria (pili)

- i. Formed of a protein cd : **pilin**
- ii. Short & thin

### Pili or Fimbriae



### 1-Ordinary pili

Adherence

the attachment resist the mucus secretion.  
Attachment of bacteria

to specific recept.on human cells

→ VF

Antigenic

### 2-Sex pili : Conjugation

Gene transfer between bacteria

# C - Endospores

## A-Definition

Highly resistant resting phase formed by *Bacillus* & *Clostridium* for protection

## B-Sporulation

### 1-Triggering & Site

In vitro : by onset of unfavorable environmental conditions

Depletion of  
nutrients

Accumulation of  
toxic metabolites

Changes in *growth requirements*  
e.g Moisture , temperature

### 2-Stages ( structure )

\* اعملى رجب - نامى جوه حبيبه  
و متخلفيش

CM invaginates : enclosing section of cytoplasm

♥ Chromosome

♥ Some ribosomes

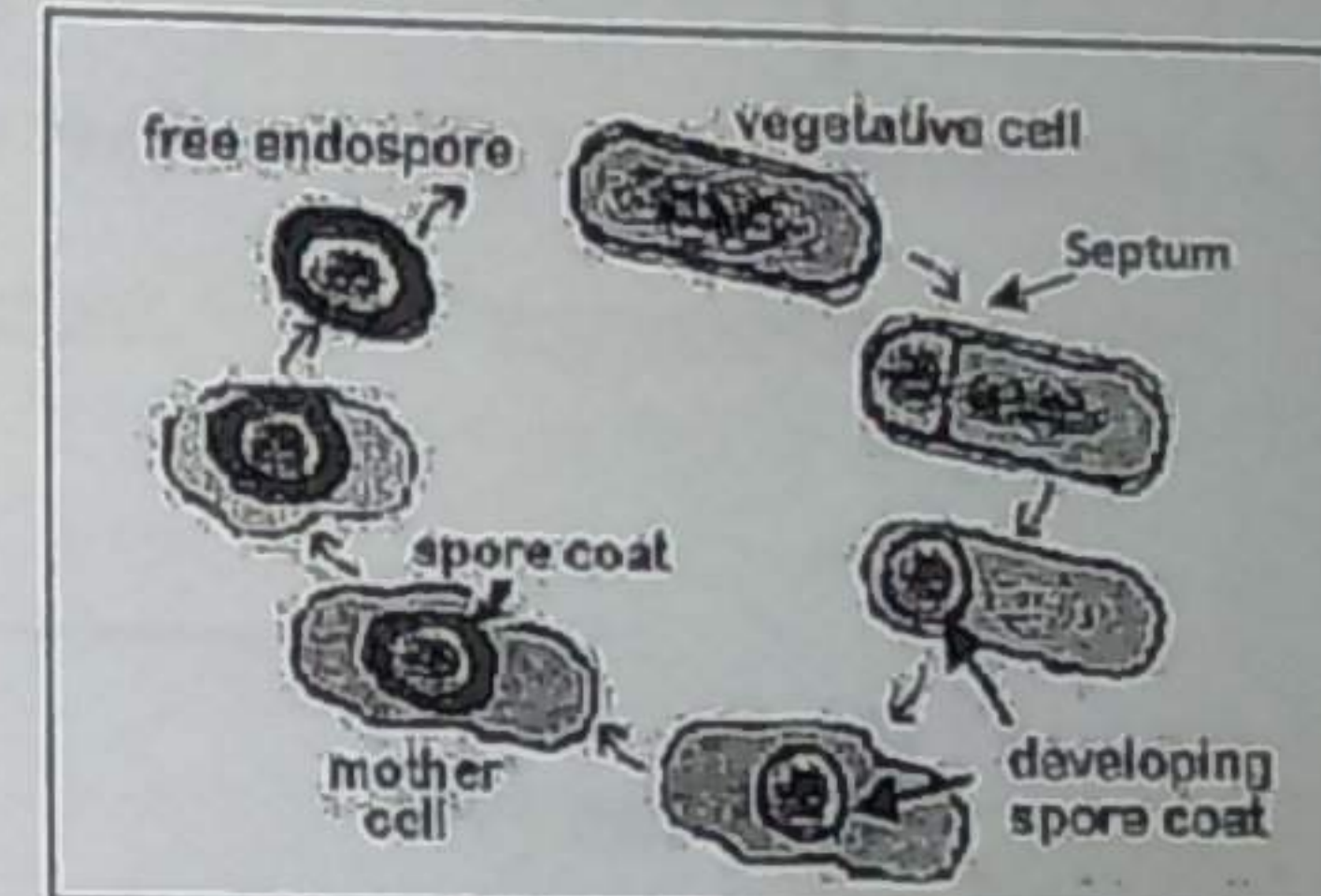
♥ Other cytoplasmic materials for germination

Thick protective covering layer

i. Exosporium

ii. Coat

iii. Cortex



### 3-Characters

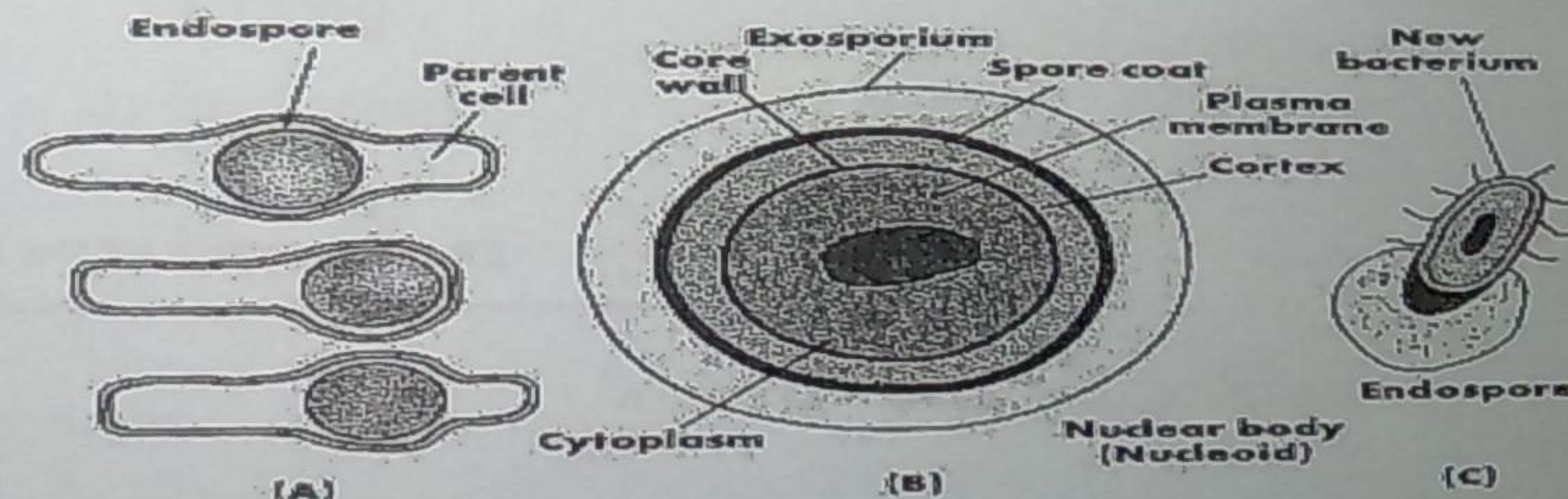
Absolute

dormancy

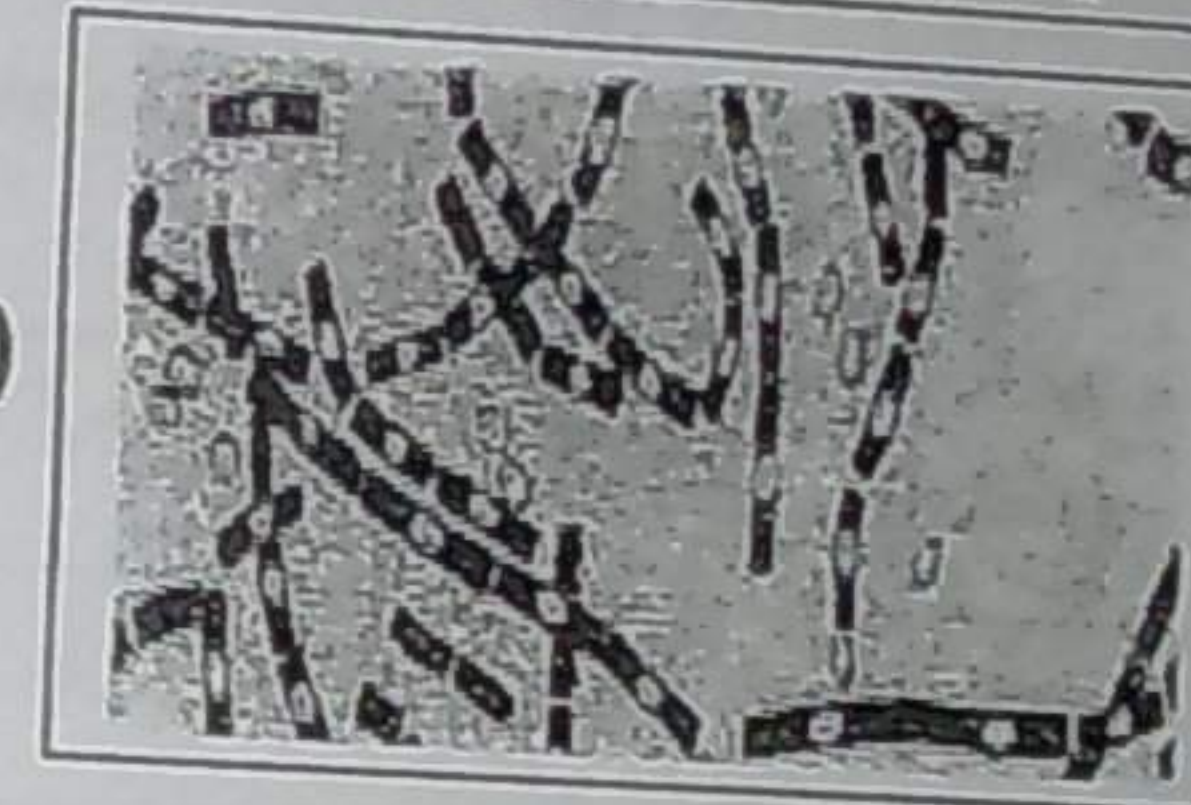
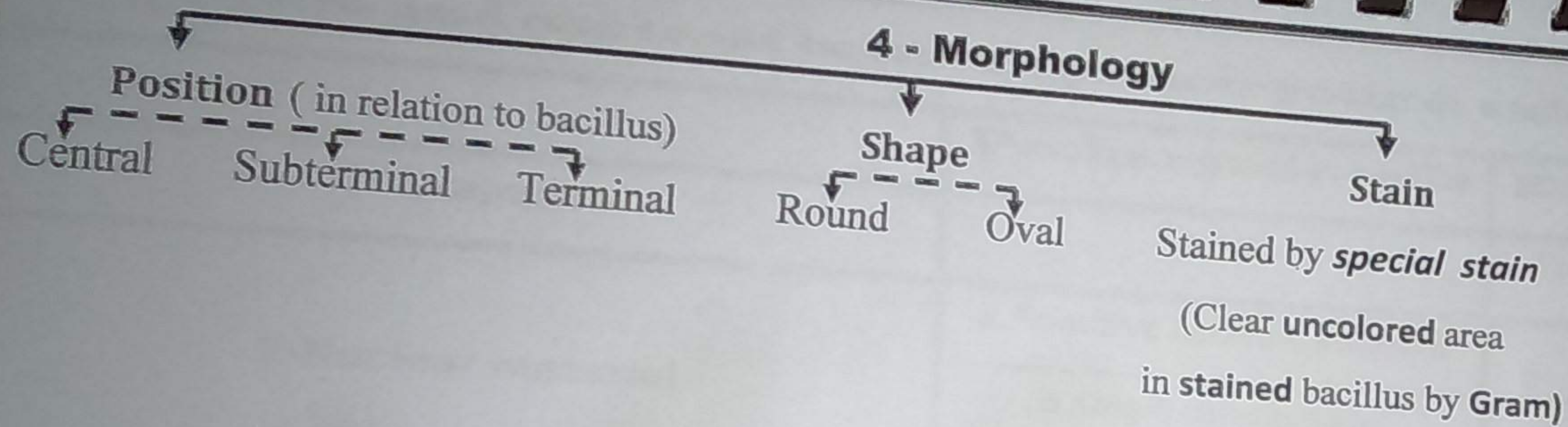
→ metabolic inactivity } نوم =

No reproduction

or growth

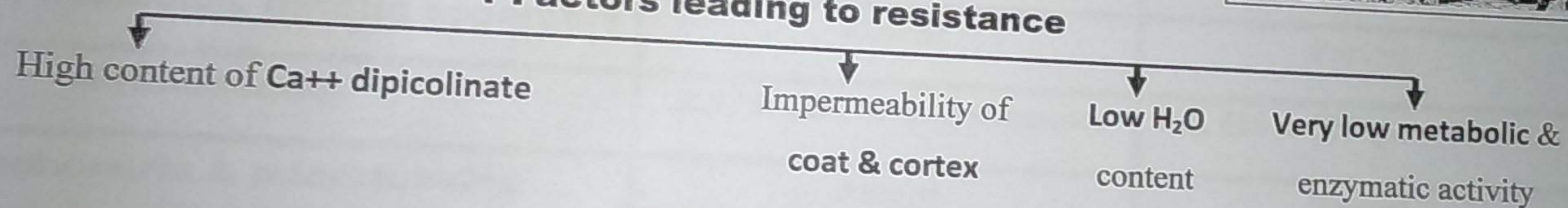


Endospore formation. A, Endospores according to their position in parent cells B, An endospore in cross-section. C, Germination of endospore

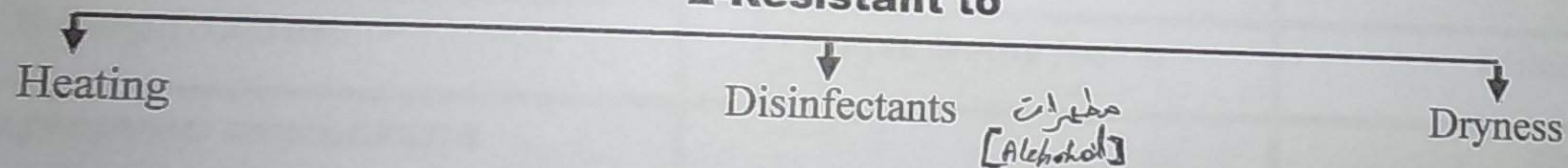


### C - Viability & resistance

#### 1-Factors leading to resistance



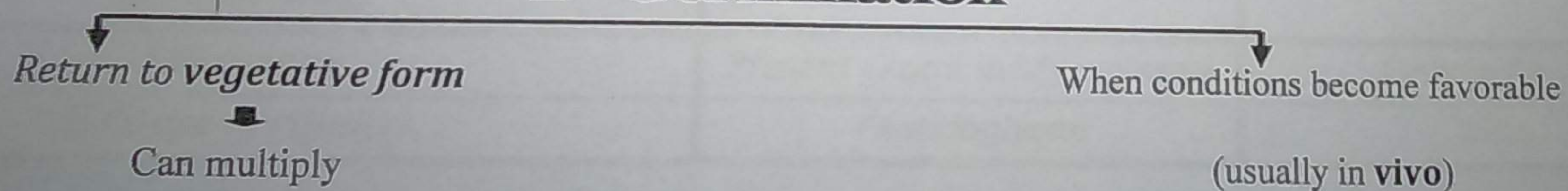
#### 2-Resistant to



#### 3-Killing

By sterilization

### D - Germination



# Compare and contrast between prokaryotic & eukaryotic cells

	Prokaryotic cells	Eukaryotic cells
1- Examples	Bacteria	Protozoa , fungi and algae
2-Nuclear material	a.Primitive nucleus: nucleoid	a.True nucleus
	b.Single chromosome	b.Multiple chromosomes
	c.No nuclear membrane	c.Nuclear membrane
3-Histones, nucleolus, mitotic apparatus	Absent	Present
4-Ribosomes	30S , 50S & 70S (polyribosome)	40S, 60S & 80S (polyribosome)
5-Mitochondria & microtubules	Absent	Present
6-Replication	?? Simple binary fission انقسام ثنائي بسيط	Mitosis
7-Cytoplasmic membrane		
i.Cholesterol	Absent except in Mycoplasma	Present
ii.Mesosomes	Present	Absent
8- Cell wall		
i.Presence	Present except in Mycoplasma	Only in fungi
ii.Cause of rigidity	Peptidoglycan	Chitin

## Essay questions on bacterial cell structure

- 1- Give a short account on peptidoglycan.
- 2- Mention functions of outer membrane.
- 3- Compare & contrast between ribosomes & mesosomes.
- 4- Compare & contrast between flagella & pili.
- 5- Compare & contrast between protein structures of bacteria (flagella & pili) → Pure Protein.
- 6 - Compare & contrast between capsule and spore regarding : site, structure & stain.
- 7- Mention 5 differences between prokaryotes & eukaryotes vivo vitro

### 8 - Give reason

- a -Spores are highly resistant.
- b-Rigidity of bacterial cell wall.
- c-Bacterial cell membrane plays a role in disease production , respiration & chemotaxis.

↳ destructive ①  
↳ Exotoxine ②

# General Bacteriology 2

Bacterial Growth

# Bacterial Growth

## Definition

↑ in size & no of individual org.

## Growth requirements

### A - Nutritional requirements

#### 1-Carbon & Nitrogen

According to C requirement, bacteria may be

##### Autotrophic

Require **inorganic** source of C as  $\text{CO}_2$  to synthesize their organic metabolites

##### Heterotrophic (parasitic) — most pathogenic

Require **organic** source of C from living host to synthesize their organic metabolites

According to N requirements

##### Exacting *كثير المطلب* → most pathogenic

Require **organic** source e.g a.a

##### Nonexacting

Require **inorganic** source e.g nitrates

#### 2-Growth factors

a.a., purines & pyrimidines

B complex vitamins & blood

#### 3-Inorganic ions (small amounts)

Phosphorus & sulfur

$\text{Ca}^{++}$  &  $\text{Mg}^{++}$

## B - Gaseous requirements

### 1-O<sub>2</sub> requirements






	1-Obligate aerobes	2-Obligate anaerobes	3-Facultative anaerobes	4-Microaerophiles
a-Growth	Only in <b>presence</b> of O <sub>2</sub>	Only in the <b>absence</b> of O <sub>2</sub>	In the <b>presence (better)</b> or <b>absence</b> of O <sub>2</sub>	Only in the presence of small amount of O <sub>2</sub>
b-Enzymes	<b>Present</b> Can degrade toxic O <sub>2</sub> Metabolites : H <sub>2</sub> O <sub>2</sub> , O <sub>2</sub> <sup>-</sup> & OH <sup>-</sup>	<b>Absent</b> Can't degrade toxic O <sub>2</sub> Metabolites: H <sub>2</sub> O <sub>2</sub> , O <sub>2</sub> <sup>-</sup> & OH <sup>-</sup>	<b>Present</b>	<b>Present</b> in small amount
i. Superoxide dismutase ii. Catalase				
c-Source of energy	<b>Aerobic</b> respiration	♥ <b>Anaerobic</b> respiration ♥ <b>Fermentation</b>	♣ <b>Aerobic</b> respiration ♣ <b>Anaerobic resp. &amp; fermentation</b>	<i>Aerobic respiration</i>
d-Examples	Mycobacterium TB	Clostridia	Most pathogenic bacteria	Campylobacter

### 5-Aerotolerant anaerobes

Grow in the presence of O<sub>2</sub> (has **superoxide dismutase**), but **don't** use it to obtain energy → **obligate fermenters**

TABLE 6.1

**The Effect of Oxygen on the Growth of Various Types of Bacteria**

	a. Obligate Aerobes	b. Facultative Anaerobes	c. Obligate Anaerobes	d. Aerotolerant Anaerobes	e. Micro-aerophiles
<b>Effect of Oxygen on Growth</b>	Only aerobic growth; oxygen required.	Both aerobic and anaerobic growth; greater growth in presence of oxygen.	Only anaerobic growth; ceases in presence of oxygen.	Only anaerobic growth; but continues in presence of oxygen.	Only aerobic growth; oxygen required in low concentration.
<b>Bacterial Growth in Tube of Solid Growth Medium</b>					
<b>Explanation of Growth Patterns</b>	Growth occurs only where high concentrations of oxygen have diffused into the medium.	Growth is best where most oxygen is present, but occurs throughout tube.	Growth occurs only where there is no oxygen.	Growth occurs evenly; oxygen has no effect.	Growth occurs only where a low concentration of oxygen has diffused into medium.

### 2-CO<sub>2</sub> requirements

Most bacteria need only CO<sub>2</sub> present in air (0.05%)  
↳ most pathogenic

Some need higher conc. of CO<sub>2</sub> (5-10%) e.g Neisseria

## C - Physical requirements

### 1-Temperature

*Psychrophilic*

Range : 5-30 C

*Mesophilic*

Range : 10-45C

e.g Pathogenic bacteria (OT:37C)

*Thermophilic*

Range : 25-80 C

### 2- Hydrogen ion concentration (pH)

*Most pathogenic bacteria*

7.2-7.6 (neutrophilic)

*Lactobacillus*

Acidic pH ( acidophilic)

*Vibrio cholera*

Alkaline pH (alkalophilic)

## Measurement of bacterial growth

**Bacterial count** : measure n= of bacteria

**Total cell count** : no of living & dead bacteria

*Dry weight*

*Turbidity*

**Viable cell count** : no of living bacteria

*No of colony forming units (CFU)*

Each bacterium multiplies → 1 colony

*Practical*

### ✓ *Generation time (doubling time)*

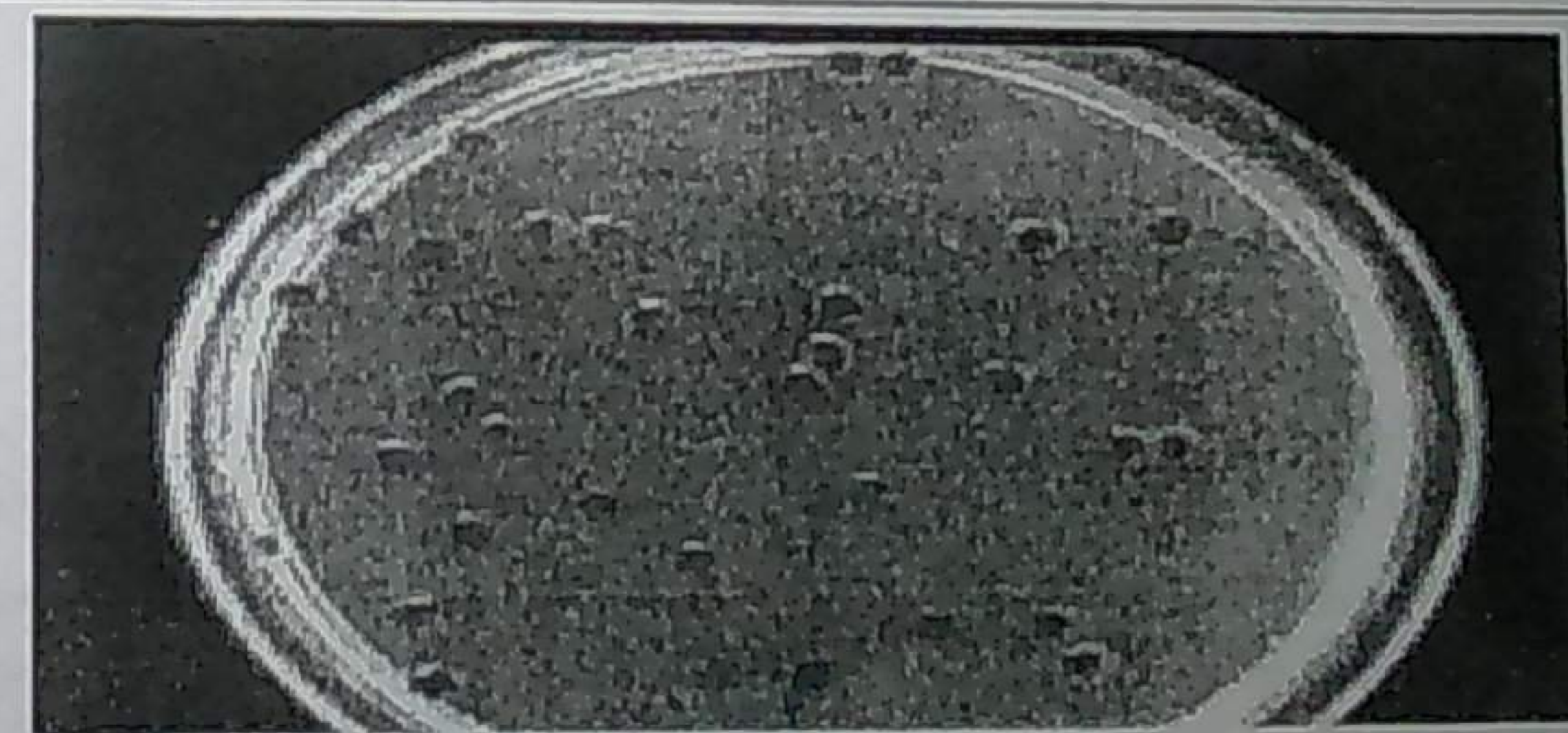
Time required by bacteria to **double its number**

( varies from one species to another )

NB Fastidious bacteria

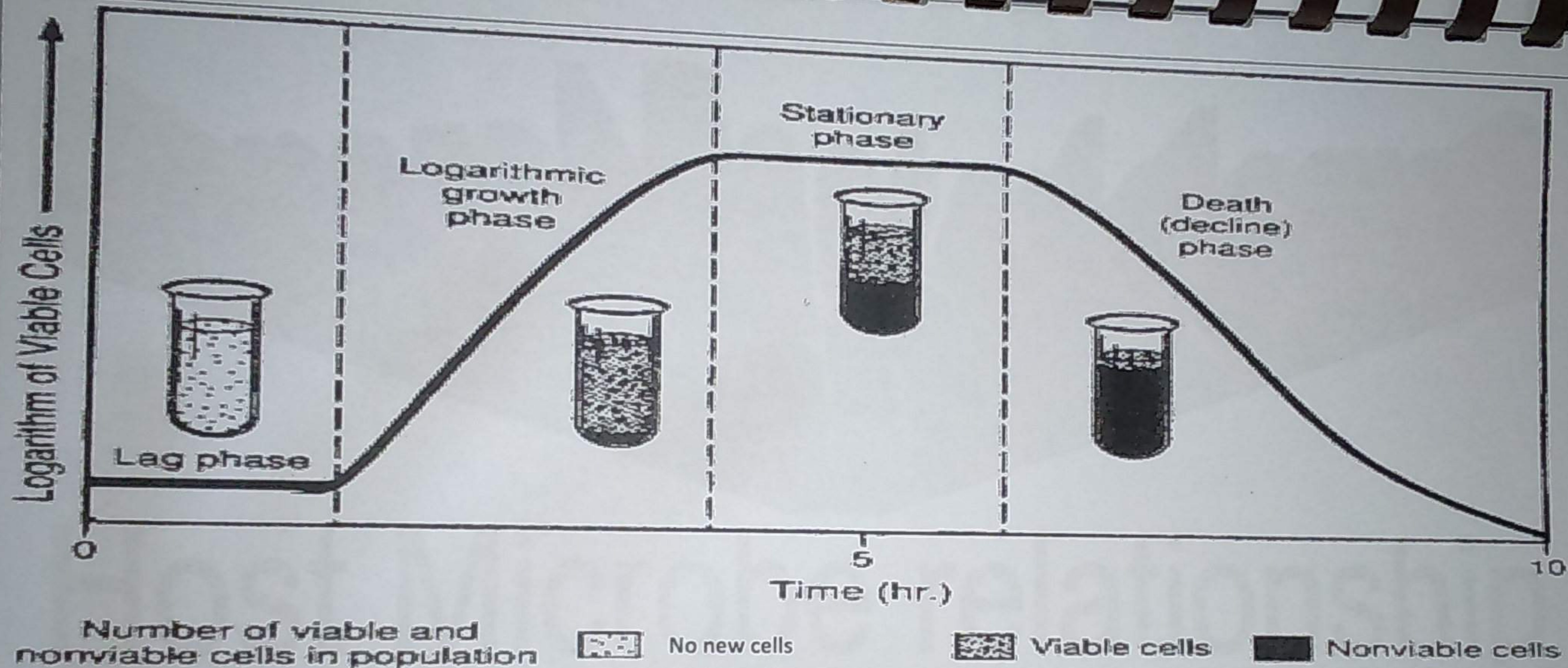
(mycoplasma)

Bacteria requiring complex nutritional requirements



# Bacterial Growth Curve

Lag phase (A)	Exponential (Logarithmic) Phase (B)	Stationary phase (C) (spore formation)	Decline phase (D)
<b>1 - Etiology</b>			
<p>a. Bacteria adapt to new medium by forming enz. for growth.</p> <p>a. Its length depends on :</p> <ul style="list-style-type: none"> <li>i- Type of org. .</li> <li>ii. Type of growth medium</li> <li>iii. Size of the inoculum  <math>\Rightarrow</math> no. of culture bacteria</li> </ul>	<p>a. Most active phase of bact.</p> <p>↓</p> <p>Highly sensitive to antibiotics</p> <p>b. This continues until:</p> <ul style="list-style-type: none"> <li>i- ↓ nutrients.</li> <li>ii- Accumulation of toxic metabolites.</li> </ul>	<p>↓ nutrients , O<sub>2</sub> starvation</p> <p>&amp;</p> <p>Accumulation of toxic metabolites</p> <p>↓</p> <p>↓ multiplication rate</p>	<p>Depletion of nutrients &amp; Maximal accumulation of toxic products</p> <p>↓</p> <p>↑ death rate</p>
<b>2 - No. of living bacteria ( Viable count )</b>			
No ↑ ( but ↑ in size)	Steady rapid ↑	Remains constant ( n of dead = n of newly formed)	Rapid ↓ ( culture may become sterile )
<b>3 - Clinical significance ( in vivo )</b>			
Incubation period of ds	Active disease ( signs & symptoms)		Convalescence & recovery



### Essay questions

- 1-Compare & contrast between obligate aerobes & anaerobes.
- 2-Give reasons :
  - Obligate anaerobes can't grow in presence of  $O_2$
  - Length of lag phase is variable
  - Decline phase of bacterial growth

# General Bacteriology 3

Host Microbe relationship

Bacterial Classification

# Host Microbe relationship

## Normal flora

A - DTT

### Definition

Org. that grow  
in healthy persons

Don't normally cause ds

### Time of acquisition

Fetus is *sterile until rupture of membrane*

Acquires flora (colonization)  
during passage in vagina

Newborn acquires more flora  
from environment : food & other humans

### Types

Resident

Found for  
prolonged time  
e.g in GIT

Transient

Found  
temporarily  
e.g in skin  
& nose

### B - Beneficial effects : ⊖ pathogenic bacteria by

Covering their  
attachement sites

Consuming  
nutrients

Production of  
toxic metabolites

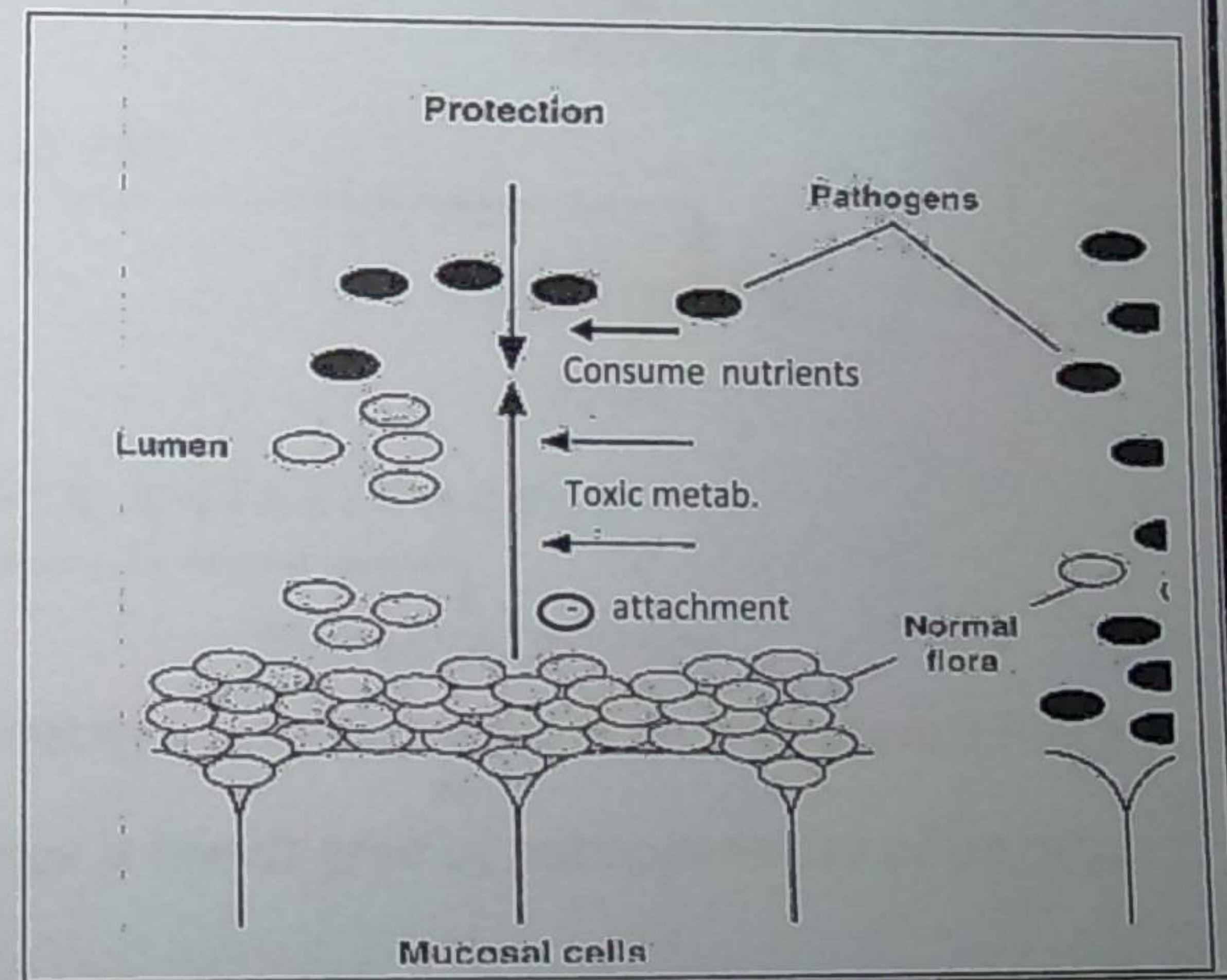
### C - Harmful effects : Opportunistic → cause ds

In immune-  
compromised  
hosts

When introduced  
outside their normal sites

Superinfection  
(E)

- ♦ E.coli of intestine
- ♦ Staph.epidermidis of skin

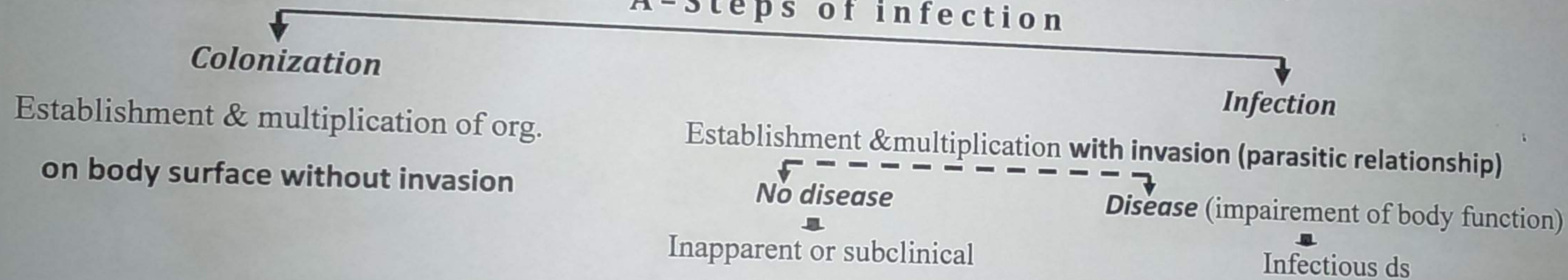


## Types of relationship between host & microbe

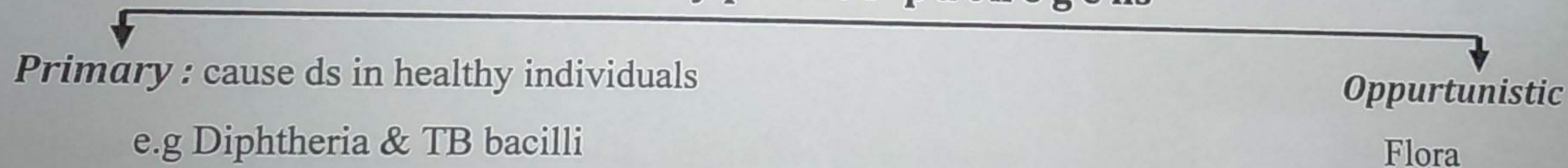
	Microbe	Host	Examples
1-Mutual	Benefits: Get food & energy from host	Benefits : gets Vit K & B	Some flora in large intestine
2-Commensal		Unharmful	Some flora in skin
3-Parasitic		Harmful	Pathogenic bacteria

## Principles of infectious diseases

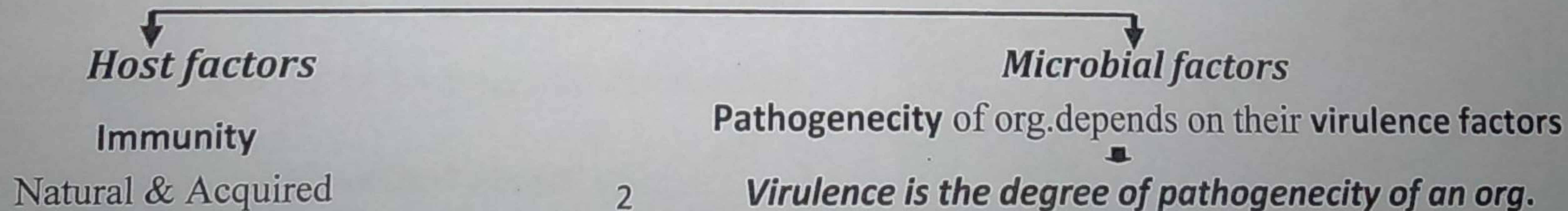
### A-Steps of infection



### B-Types of pathogens



### C-Factors affecting host parasite relationship



# Bacterial virulence factors

Structure or product that enables bacteria to cause ds

I-Adherence to host cell →

Resistance of physical removal →

Colonization

Fimbria (pili)

Adhere to receptors on

GIT & urinary epithelium

RBCs

Non fimbrial adhesins in CW

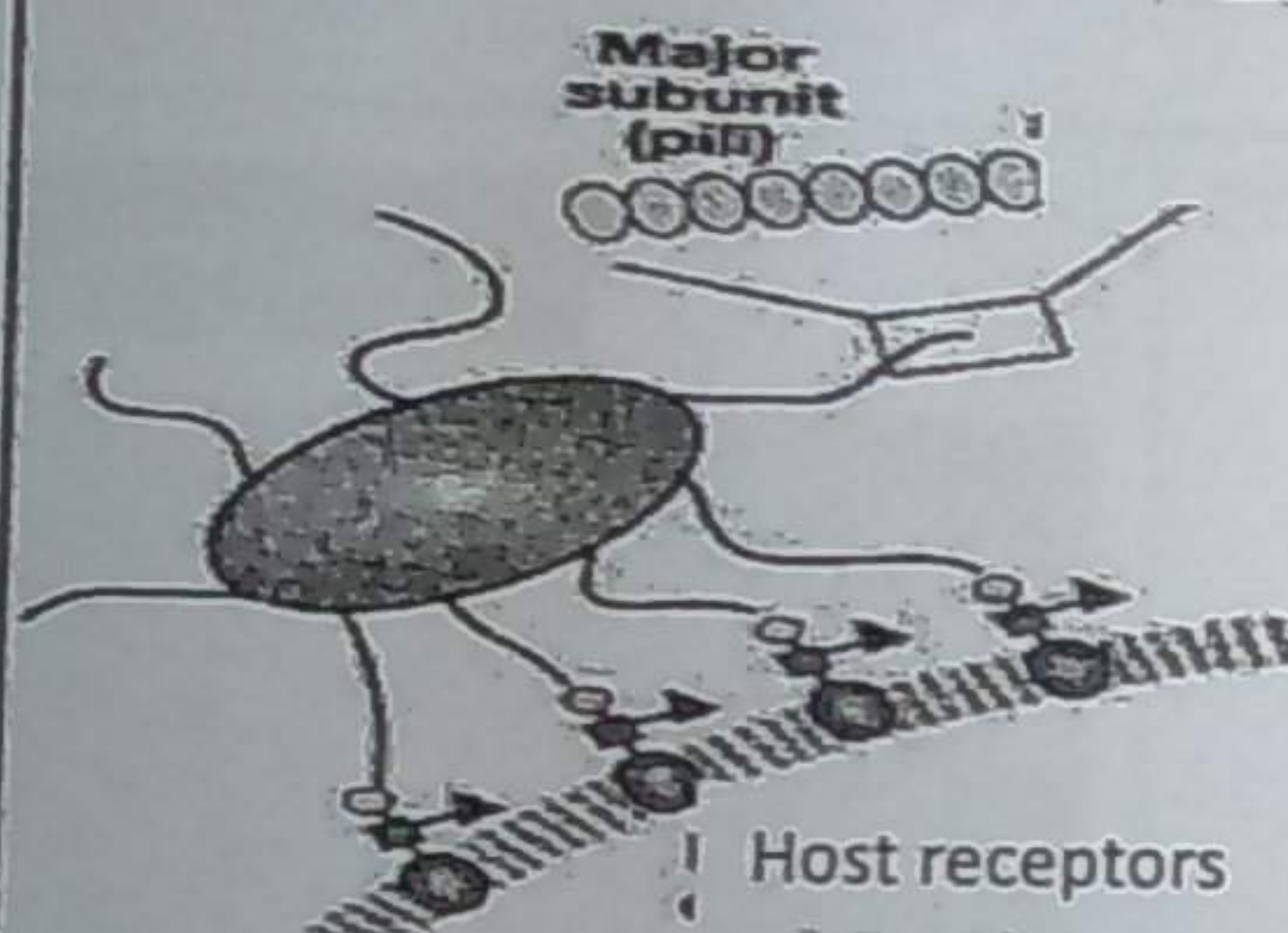
Bind to specific receptors

on host cell surface

Glycocalyx

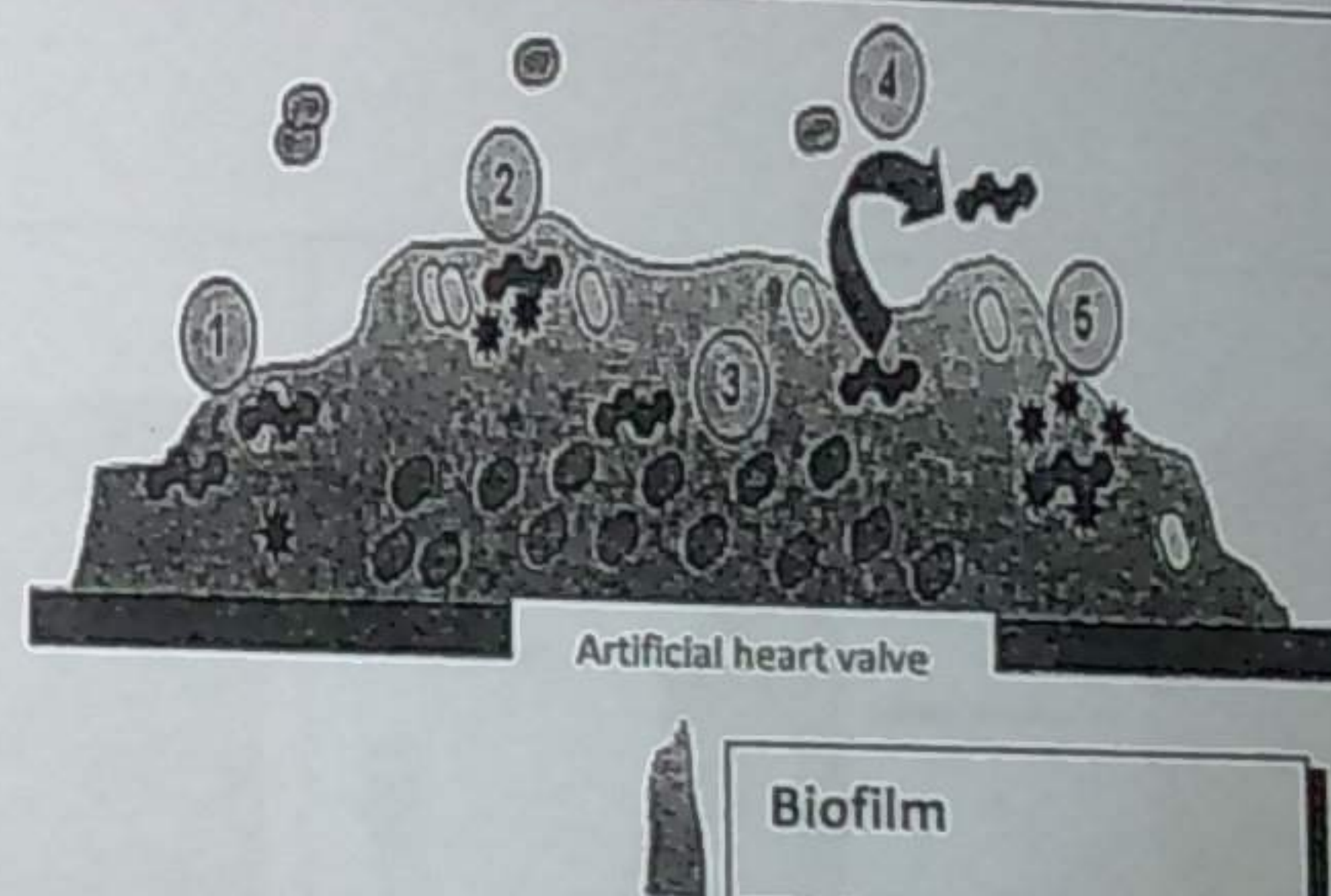
Biofilm formation

a) Pili or Fimbriae



b)

Non fimbrial adhesins



## II-Invasion of host cells

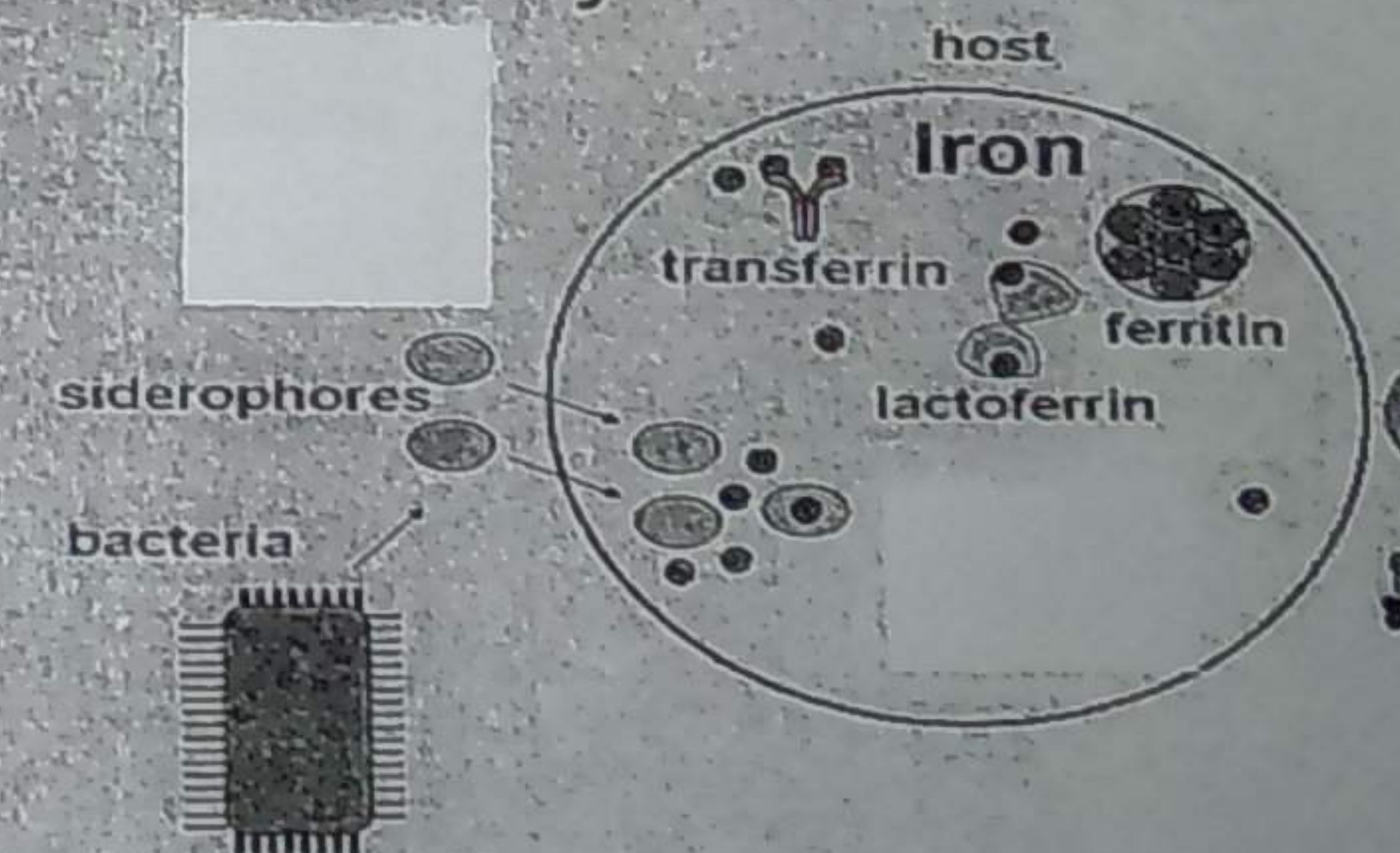
Entry in tissues → Multiplication → Spread to other tissues

## III-Competition for human iron by Siderophores

Iron chelators excreted by bacteria into environment

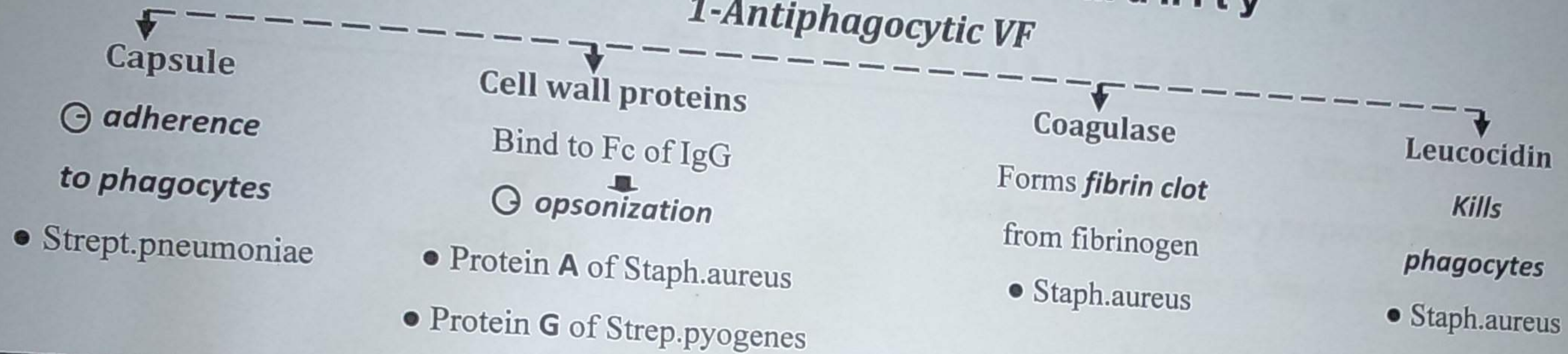
Bind iron → re-enter the bacterial cell

## Iron thievery

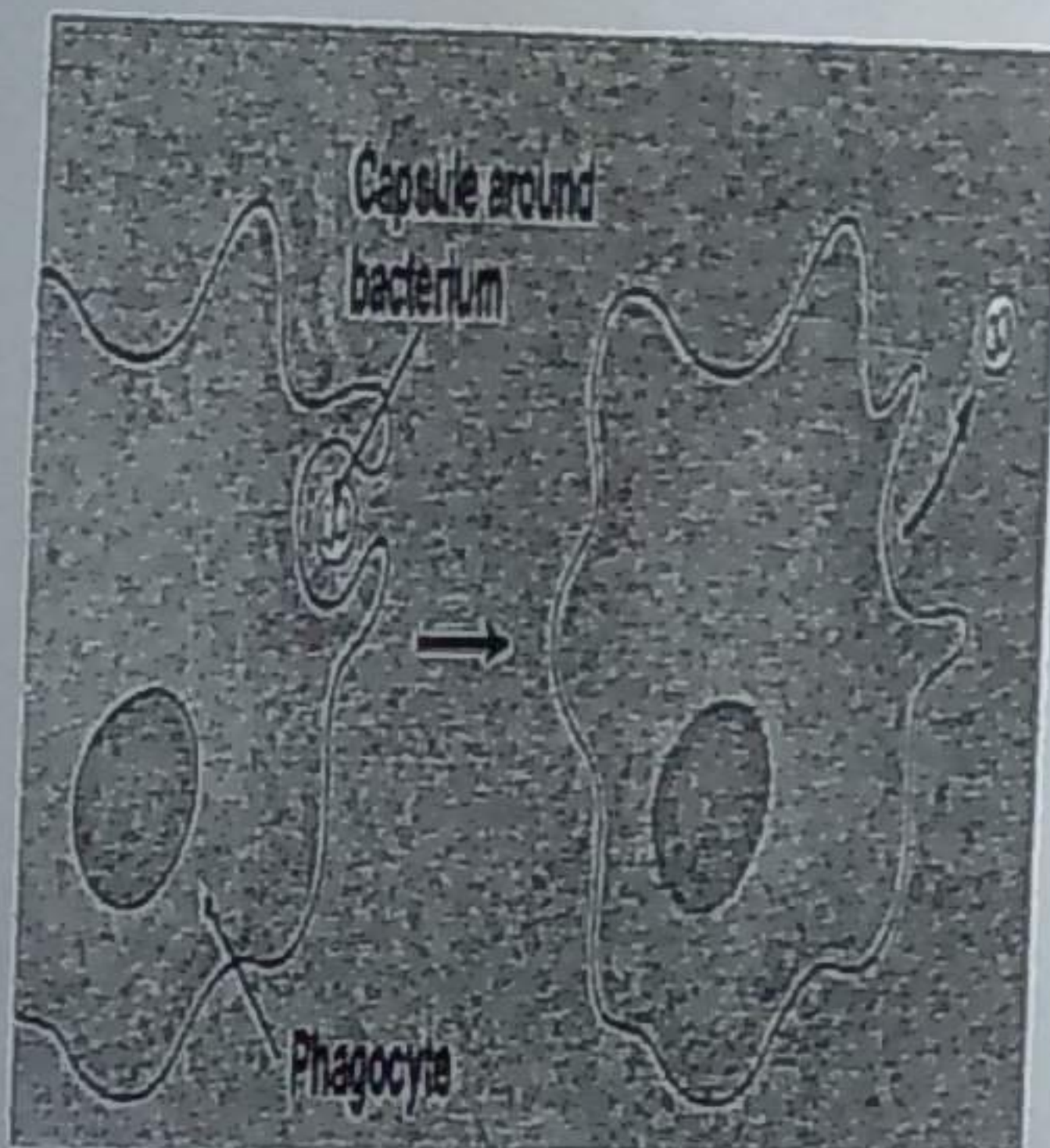


## IV - Resistance to immunity

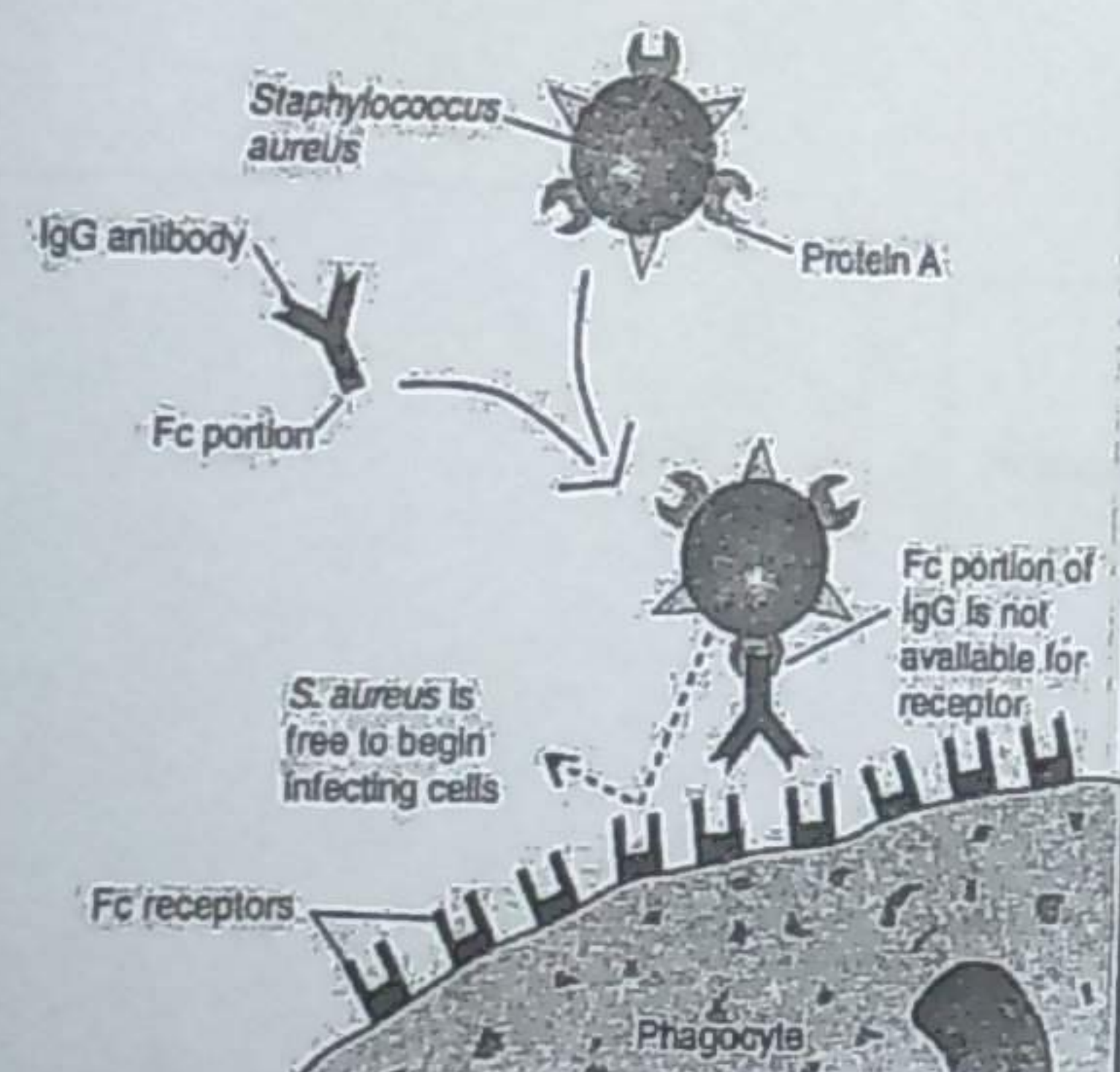
### 1-Antiphagocytic VF



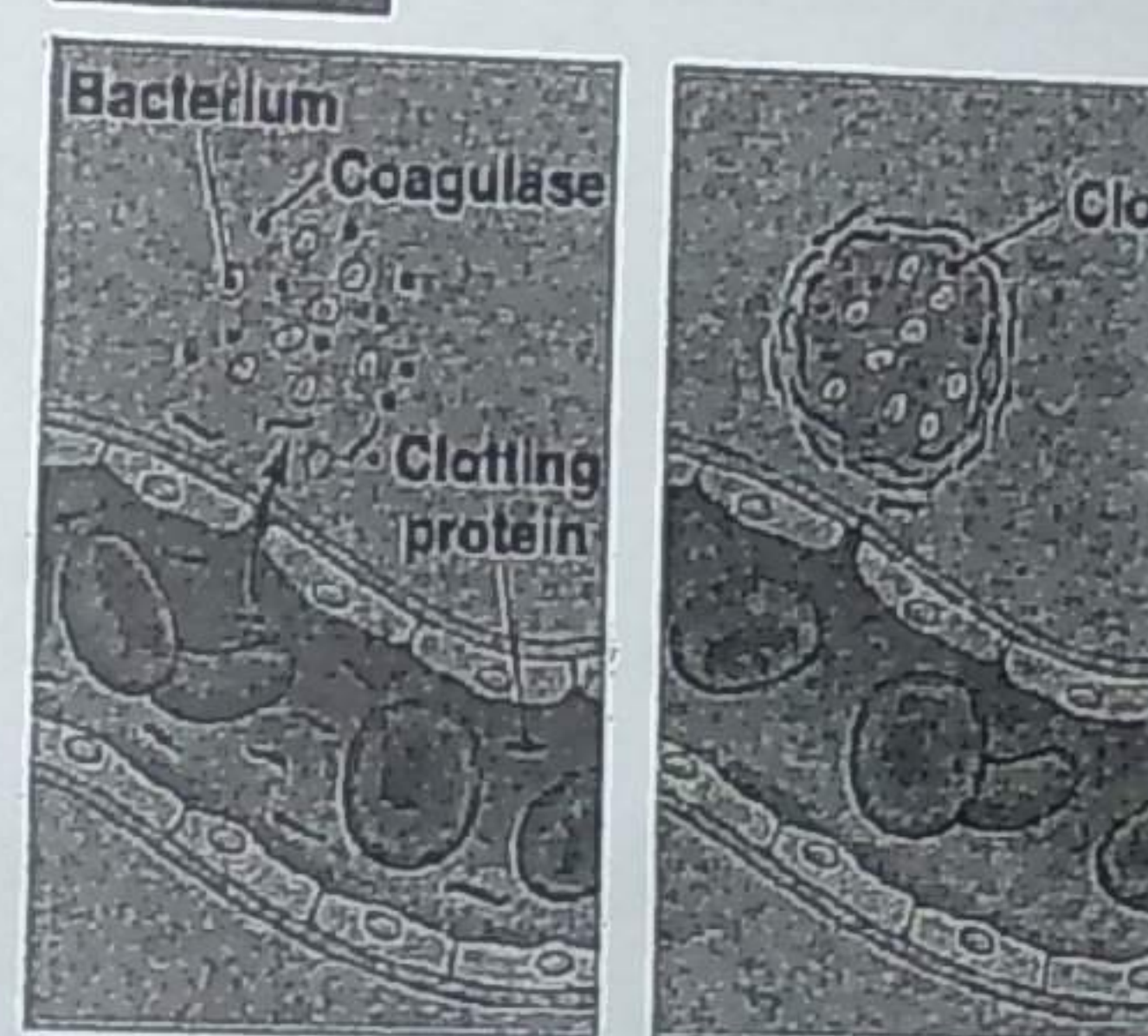
Phagocytosis blocked by capsule



fusion required for reproduction or display

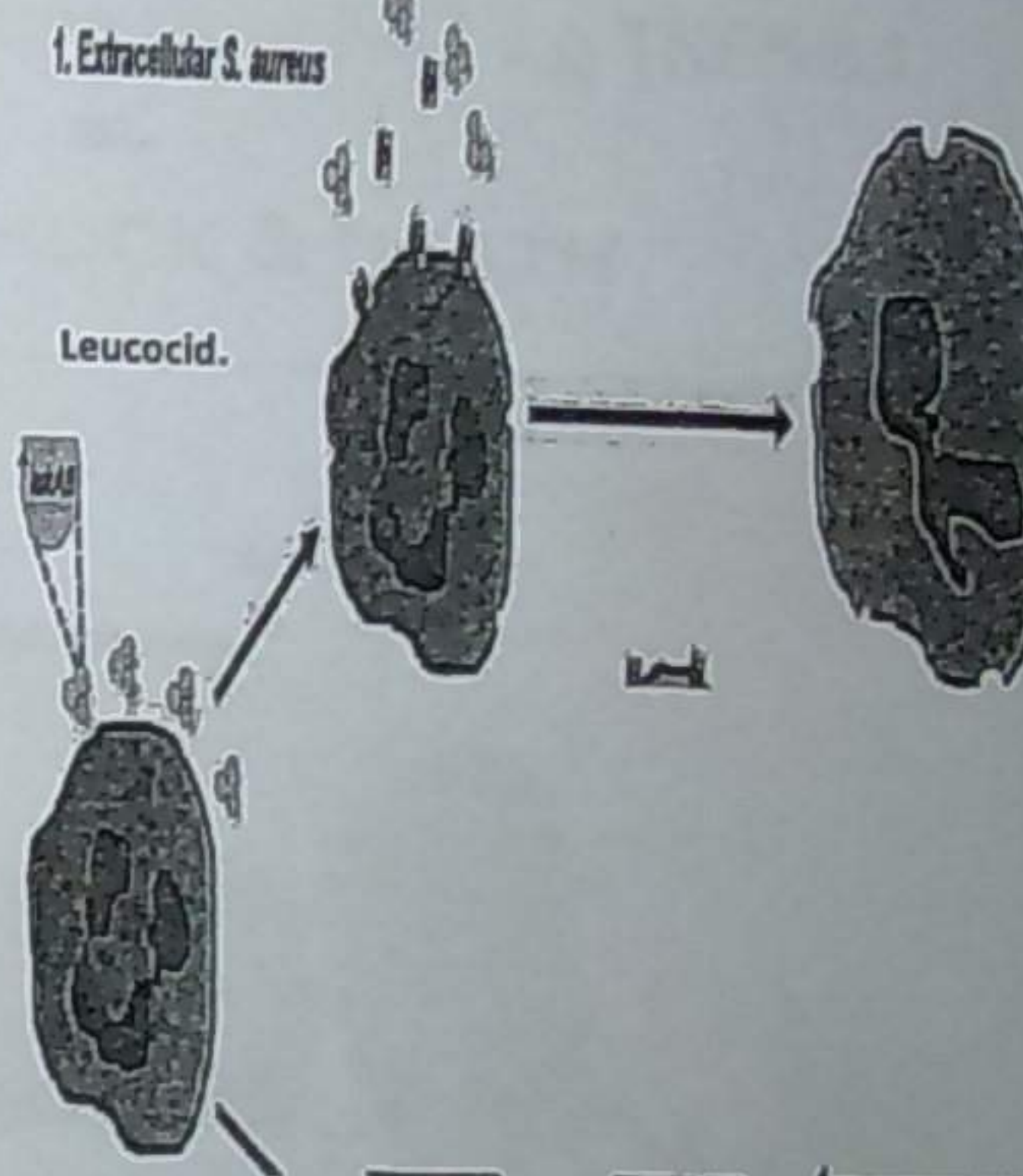


Coagulase



Bacteria produce coagulase.

Clot forms.



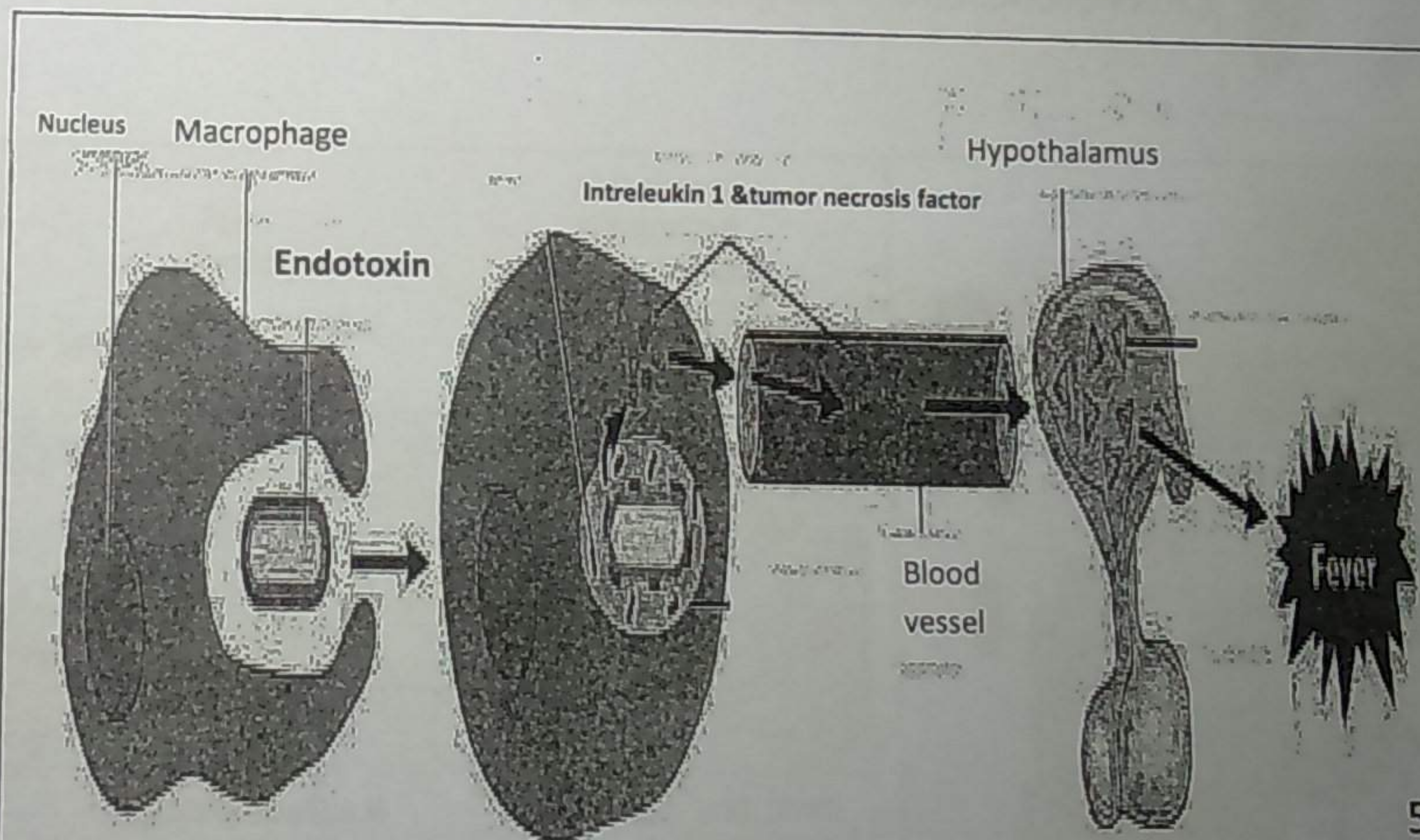
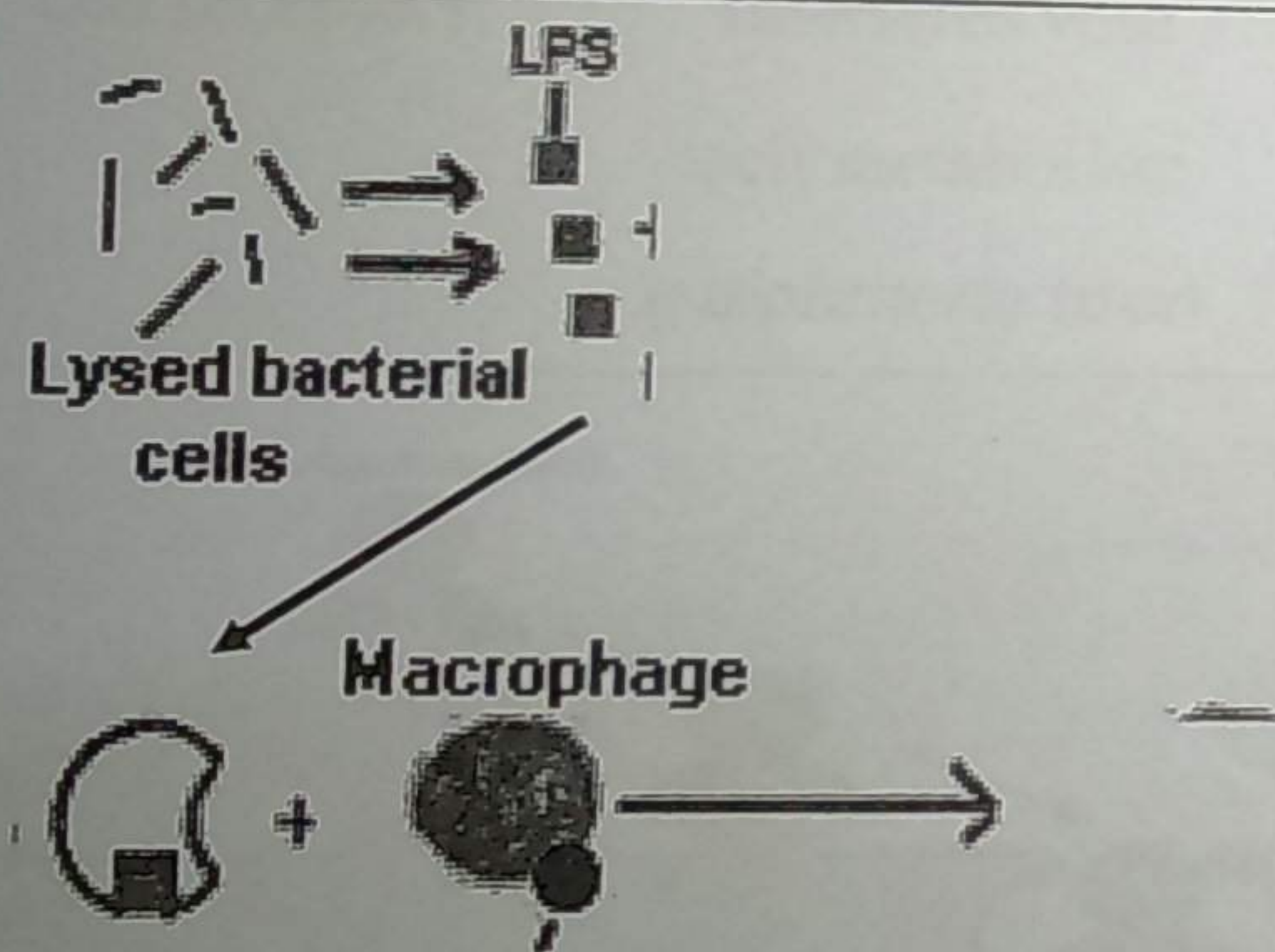
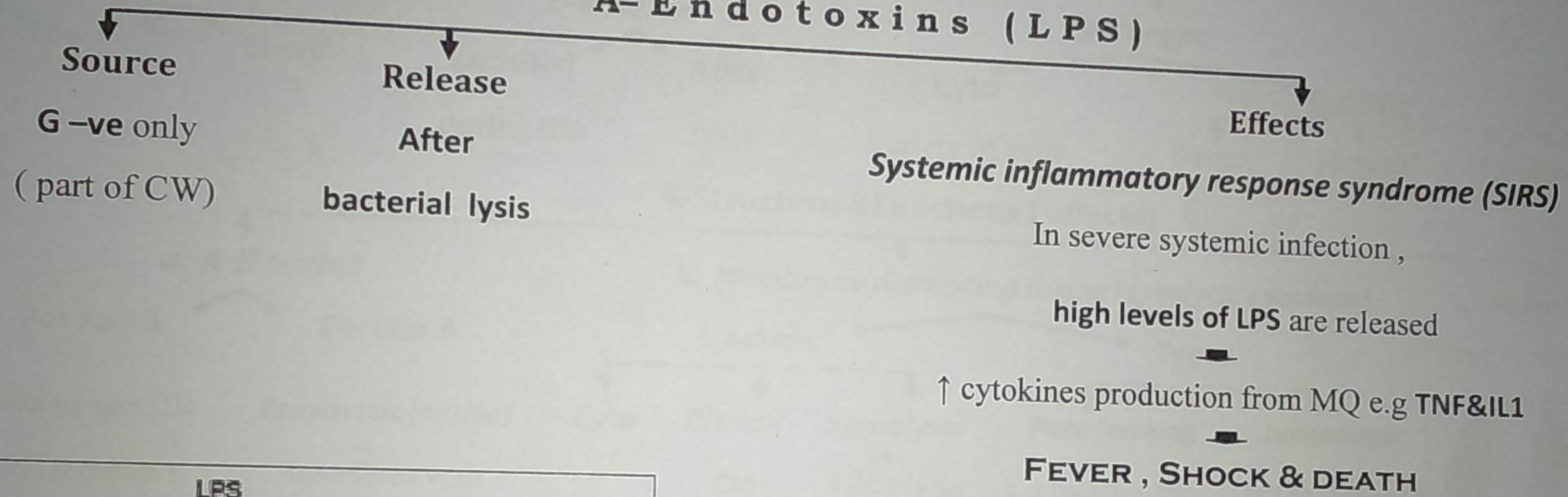
### 2-IgA protease

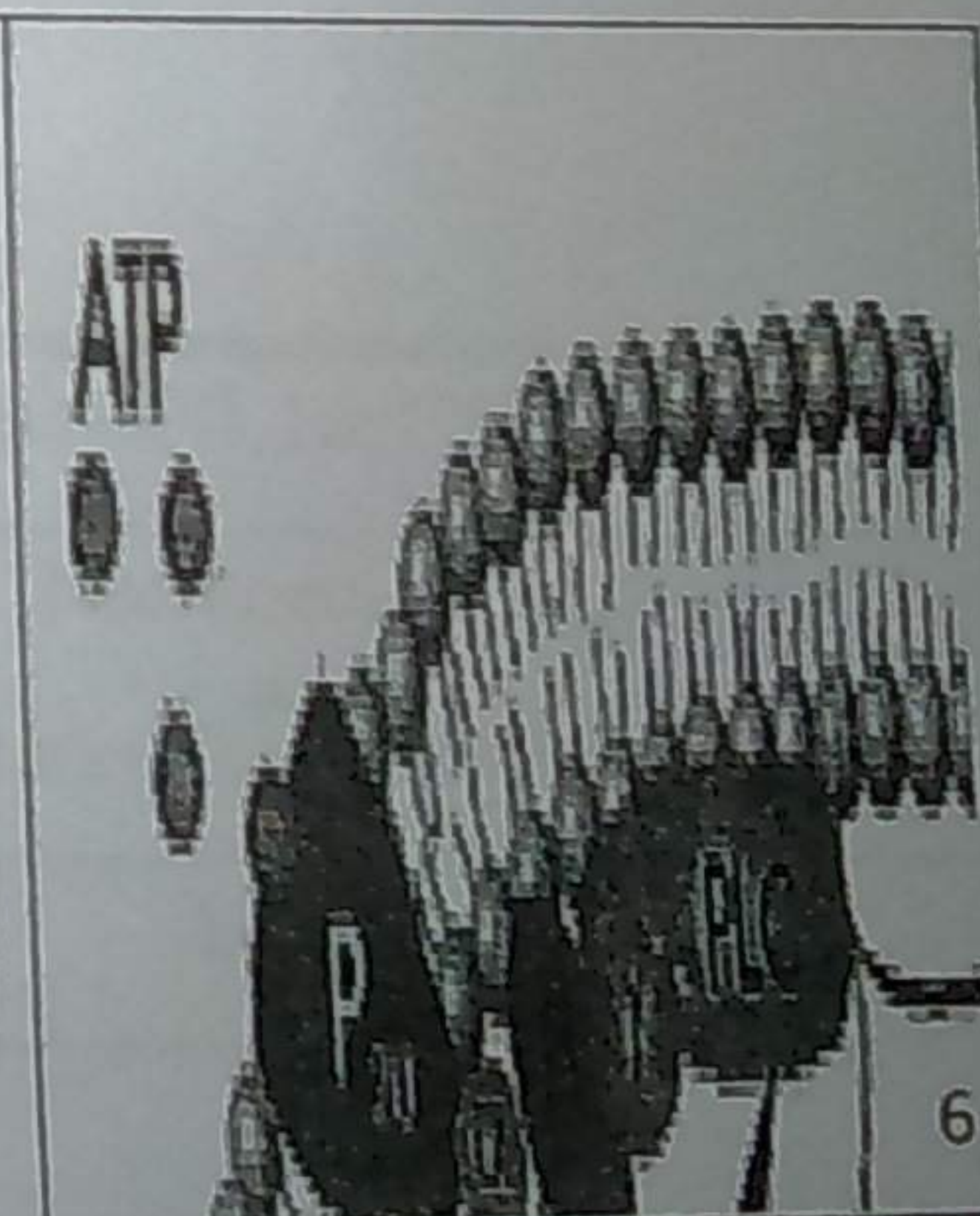
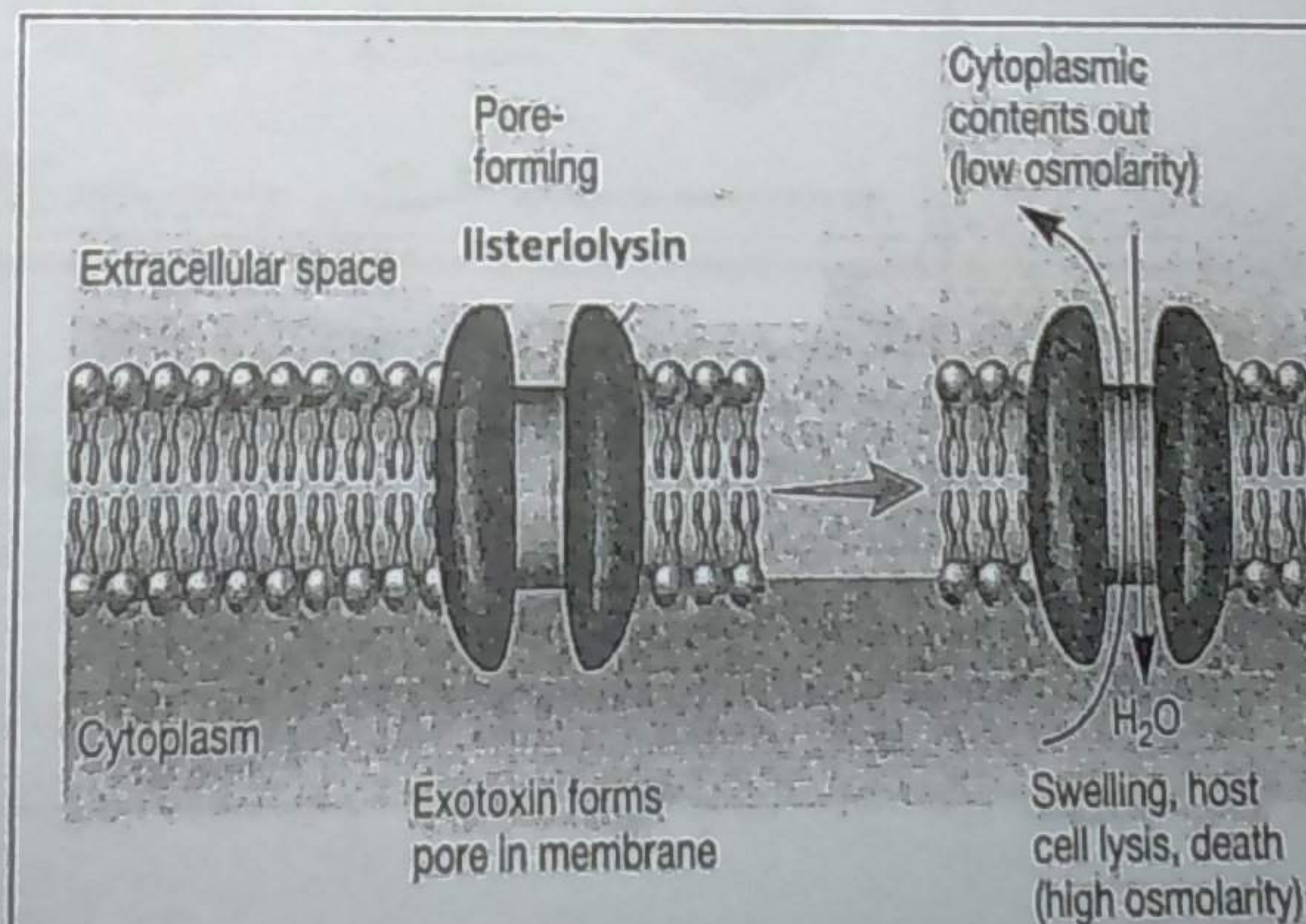
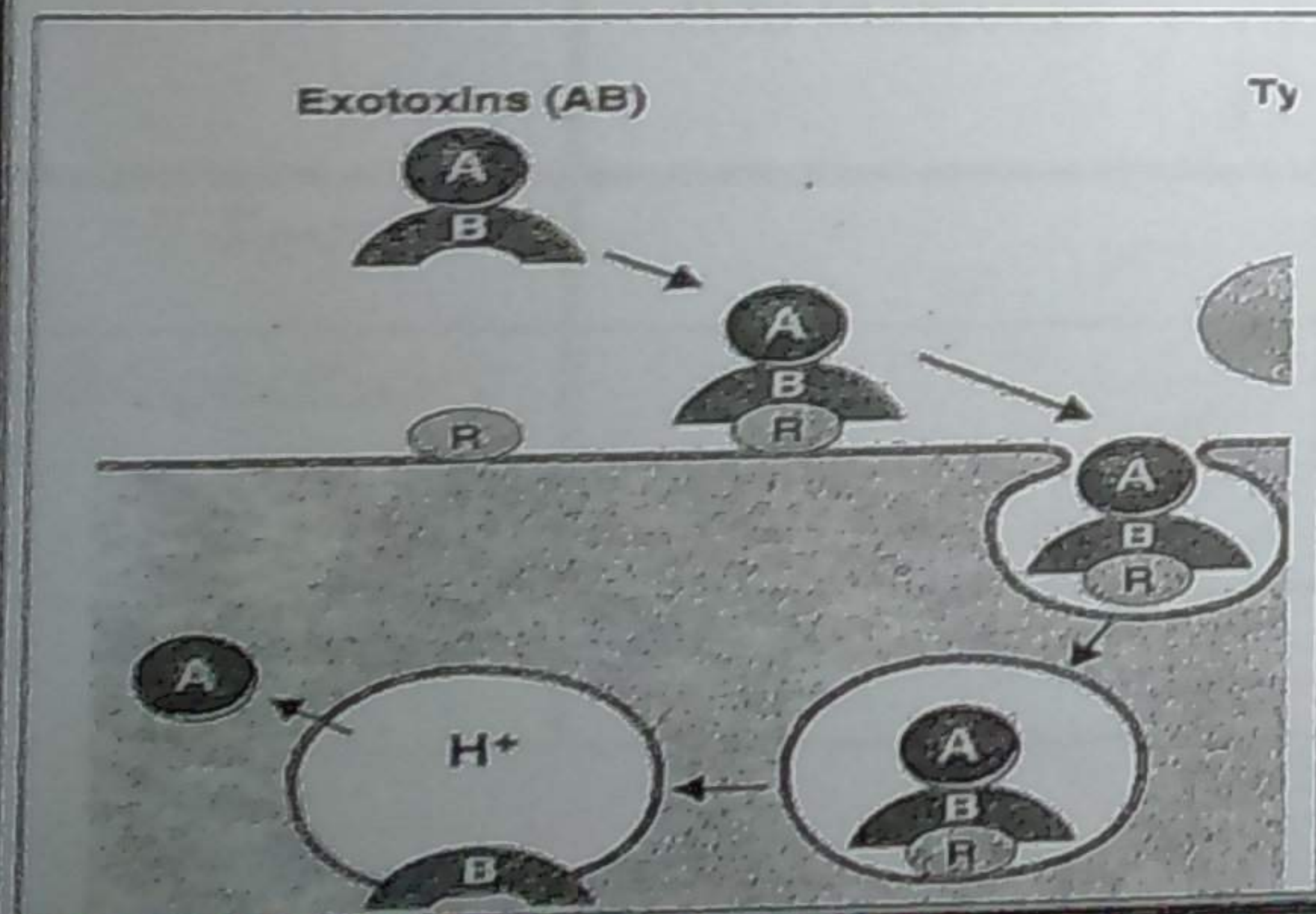
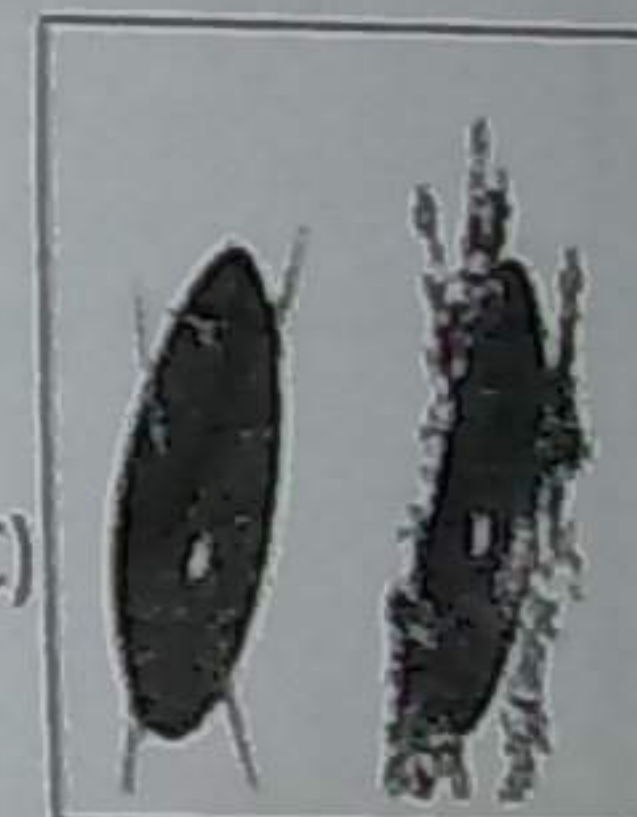
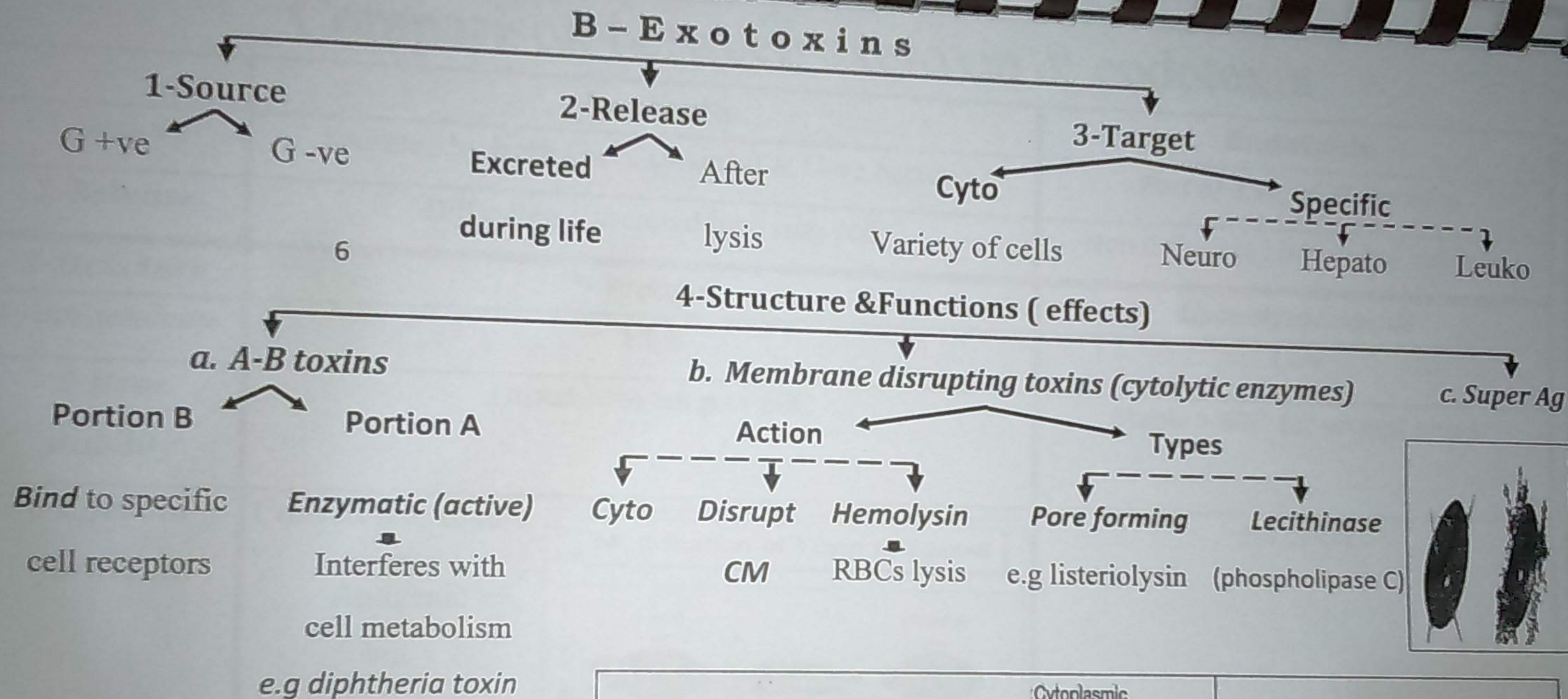
Degrades IgA → **Adherence** to mucosa

- *Strept.pneumoniae*

# V - B a c t e r i a l   t o x i n s

## A - E n d o t o x i n s ( L P S )

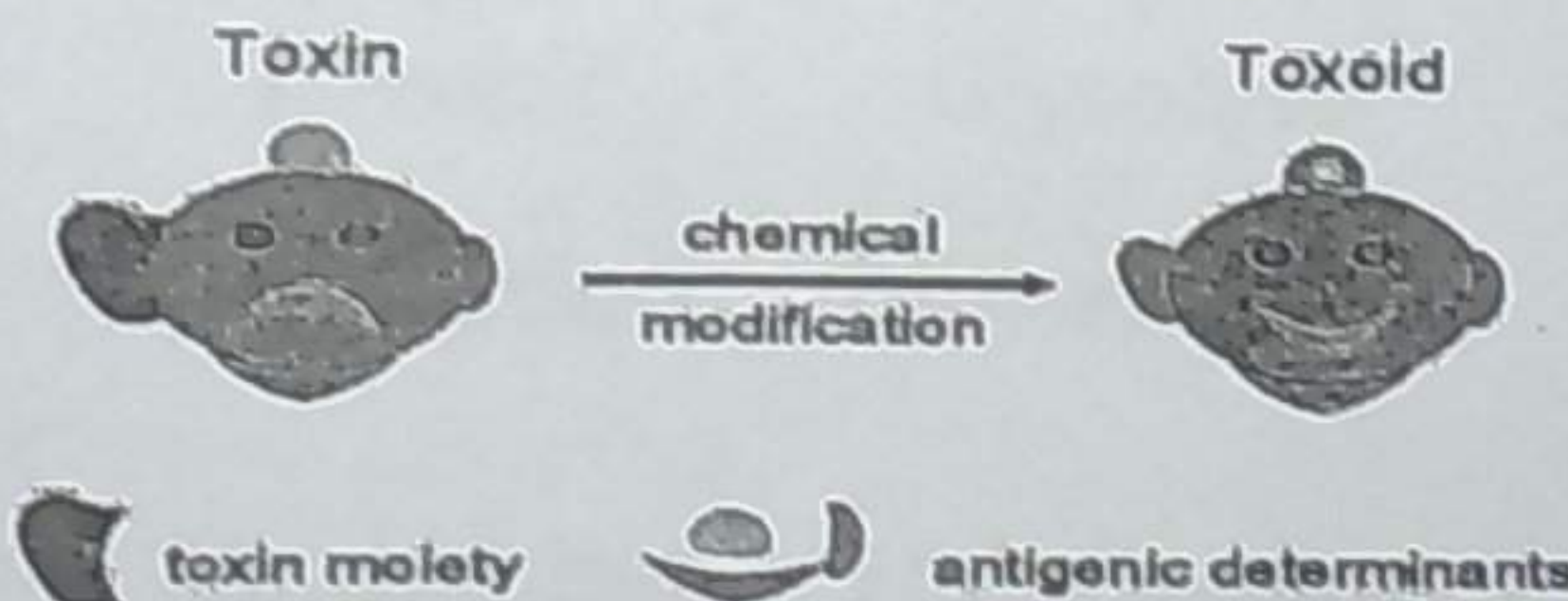




# Comparison between exotoxin & endotoxin

	Exotoxin	Endotoxin
1-Source	Secreted by <b>both</b> G+ve (mainly) & G-ve bacteria	<b>Part of CW of G -ve only</b>
2-Release	<b>Diffusible</b> : secreted by living cell	Non diffusible : released on cell lysis
3-Structure	<b>Protein</b>	<b>Lipopolysaccharide</b>
4-Antigenecity	<b>High</b>	<b>Low</b>
5-Heat stability	Unstable to temp. > 60C	<b>Stable &gt; 60C</b> for several hours
6-Effect of formalin	Convert it to <b>toxoid</b> ↓ <b>Antigenic</b> but <b>non toxigenic</b>	No effect
7-Fever	No	<b>Yes</b> , by release of IL1 & TNFα from MQ
8-Specificity	<b>Specific</b>	Non specific All cause fever & shock
9-Toxicity	<b>Very high</b>	<b>Low</b>

## Modification of Toxin to Toxoid



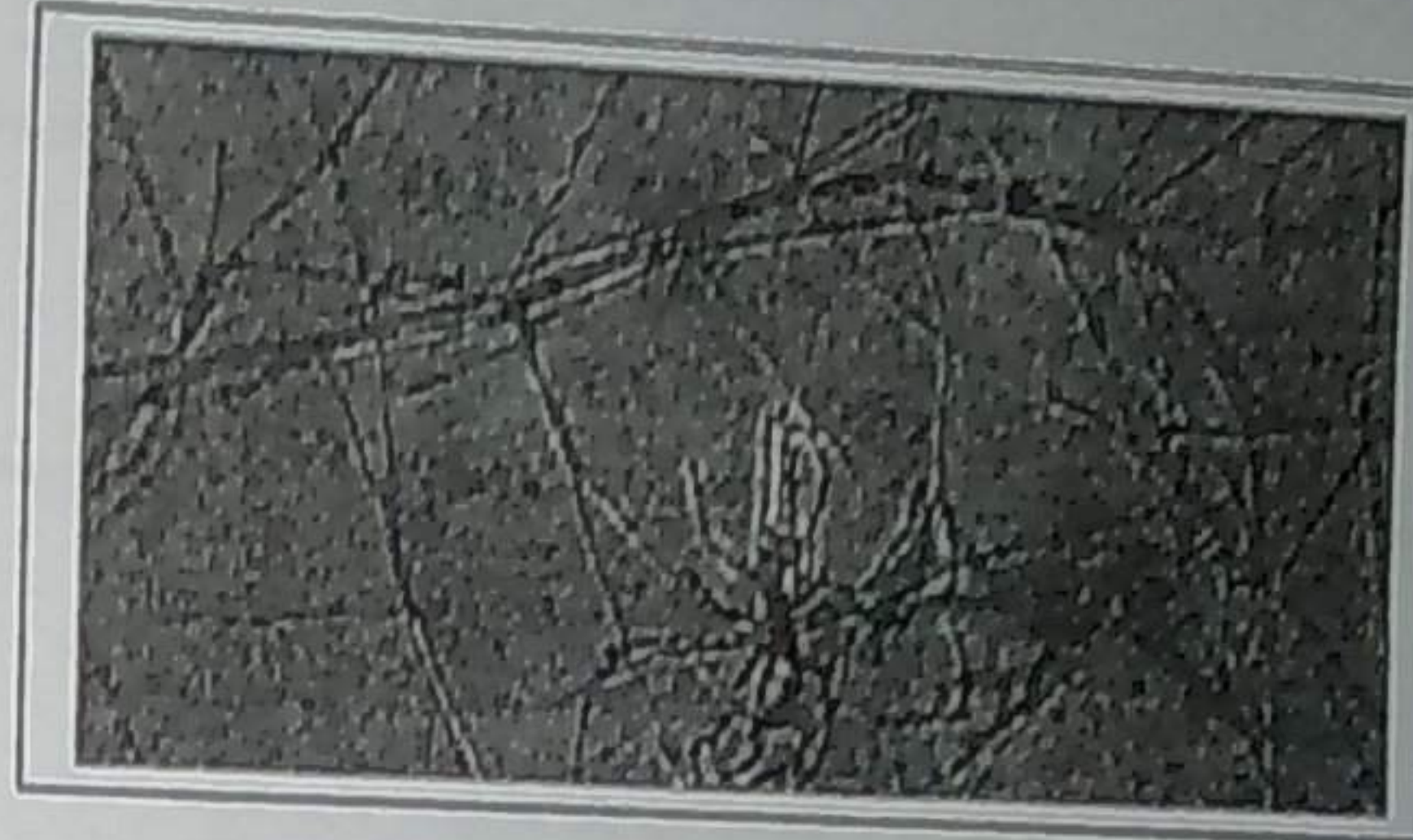
# Bacterial Classification

## I- Old system:

Based on *phenotypic characters*

### A-Higher bacteria :

**Actinomyces** → Filamentous branching



### B-Lower bacteria : simple unicellular org.

#### 1-Reaction to Gram stain

- i. G+ve : violet
- ii. G-ve : pink

#### 2-Shape

- i. Cocci
- ii. Bacilli
- iii. Vibrio
- iv. Spirilla

#### 3-Nutritional requirements

#### 4-Methods of

##### energy production

- Aerobic respiration
  - For aerobes
- Glycolysis (fermentation)
  - For anerobes

#### 5-Pathogenecity

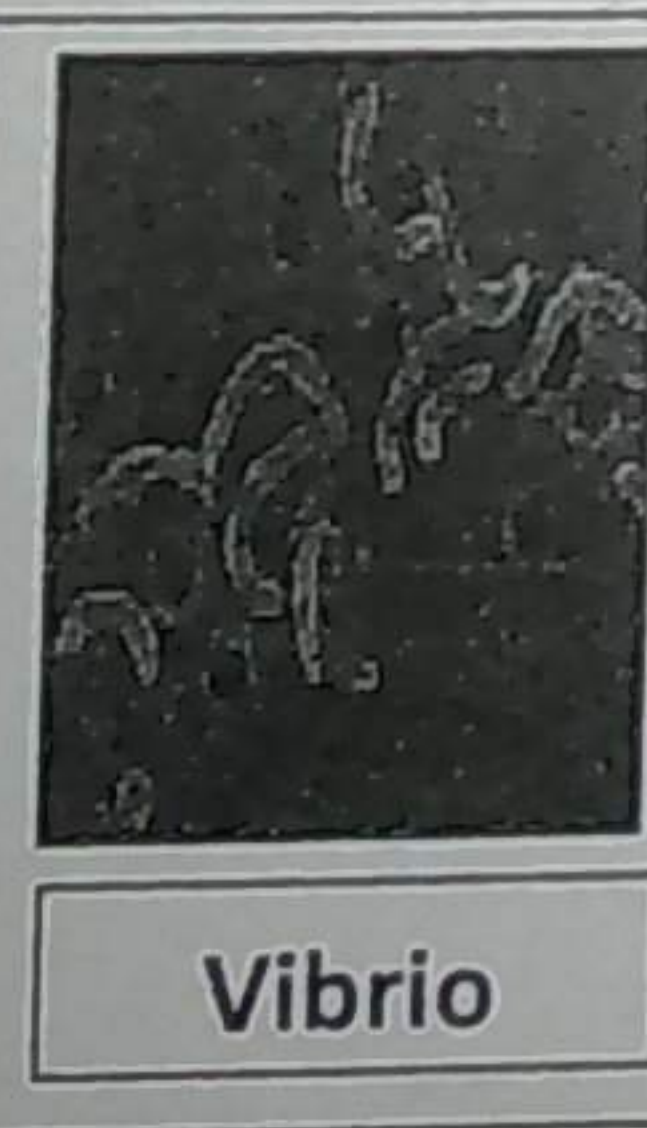
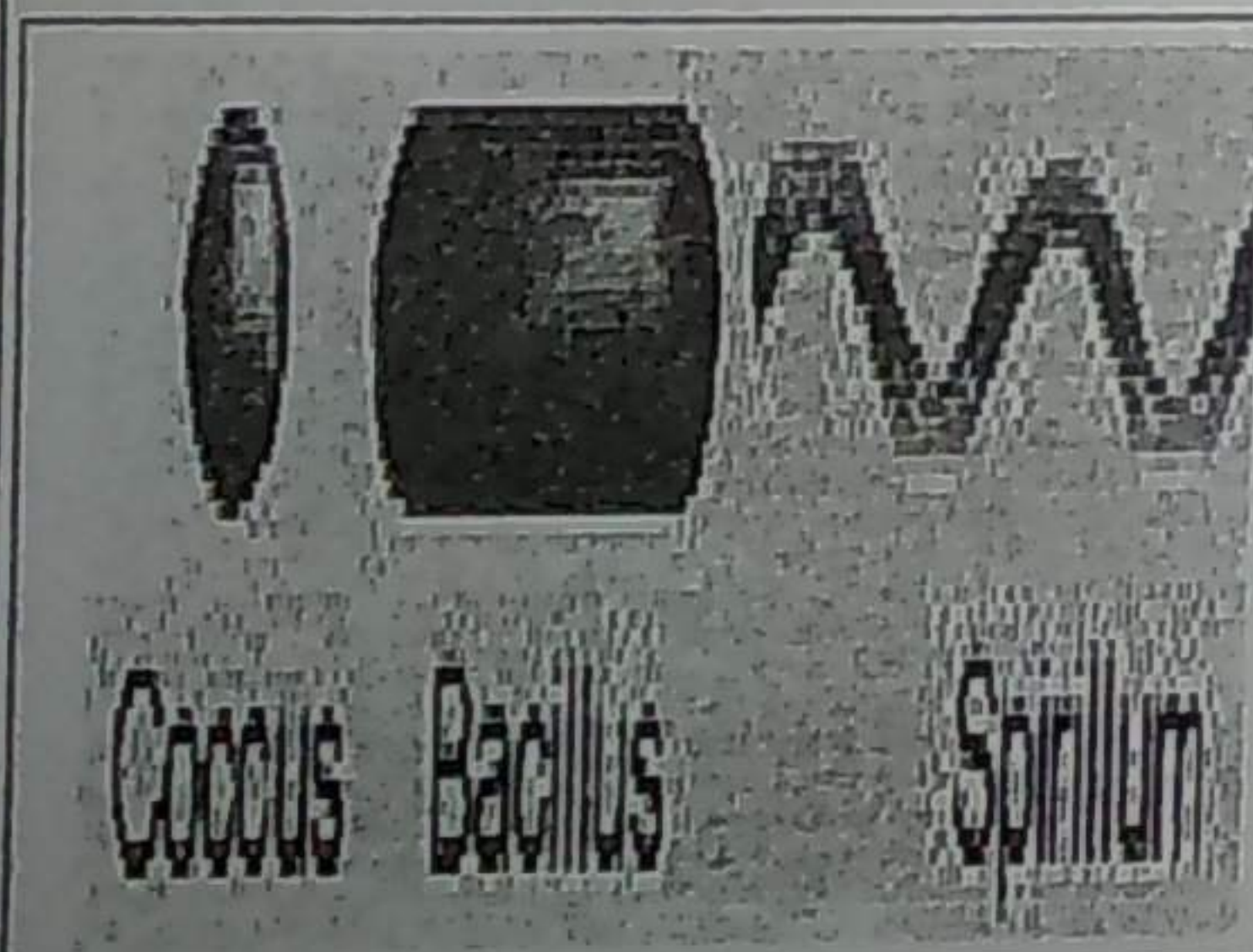
##### Saprophytes

Live on dead materials  
soil, water, dust  
No disease

##### Parasitic

Live in body of living creatures

1ry pathogens      Oppurtunistic flora



## II-New system

Based on *genotypic characters* → determine *bacterial relatedness*

	Nucleotide base composition	Nucleic base homology	Genome sequencing
Principle	Molecular % of G+C in the total DNA	Homology of DNA base sequences	Nucleotide base sequence analysis of rRNA genes
Significance	<p><i>Fixed</i> in strains of the <i>same species</i></p> <div data-bbox="693 1103 1226 1365"> <math display="block">GC \text{ content} = \frac{G+C}{G+C+T+A} \times 100</math> </div>	<p>Mixture of DNA from <i>2 related species</i> produce <i>hybrid pairs</i></p> <div data-bbox="1272 1046 2105 1815"> <p>Organism A DNA</p> <p>Organism B DNA</p> <p>① Heat to separate strands.</p> <p>② Combine single strands of DNA.</p> <p>③ Cool to allow renaturation of double-stranded DNA.</p> <p>④ Determine degree of hybridization.</p> <p>Complete hybridization: organisms identical</p> <p>Partial hybridization: organisms related</p> <p>No hybridization: organisms unrelated</p> </div>	<p>Determine <i>evolutionary relationships</i> among bacteria</p> <div data-bbox="2159 1103 2725 1328"> <p>Genes: 16S rRNA</p> </div>

				Ancestor ( from thousands of yrs )	
				Same sequence of gene coding .....	
Genus Staphylococci				Mixture of DNA produce .....	
Staph.aureus					
Strain 1	Strain 2		Strain 3	Staph.epidermidis	
Same .....					

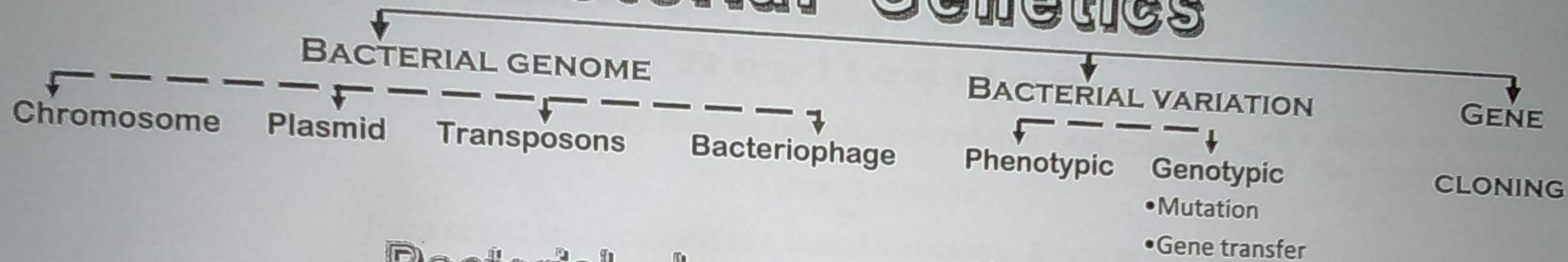
### Essay Questions

- 1- Compare & contrast between exotoxins & endotoxins.
- 2- Give a short account on membrane disrupting toxins.
- 3- Give reasons :
  - a. Normal flora are opportunistic pathogens.
  - b. Normal flora are considered as part of host immunity.
  - c. New system of bacterial classification determines bacterial relatedness
  - d- How meningococci can cause septic shock ?

General Bacteriology 4

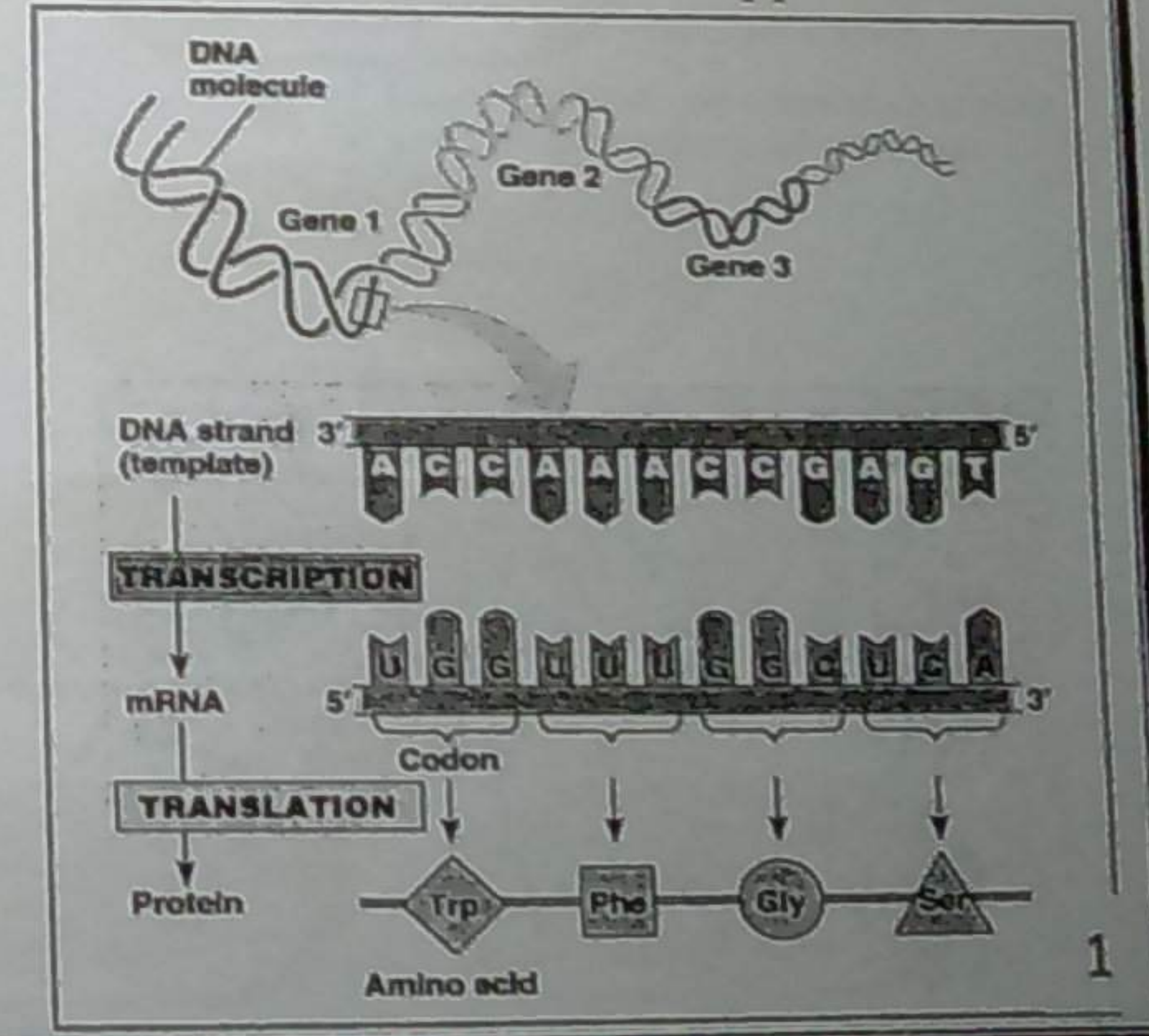
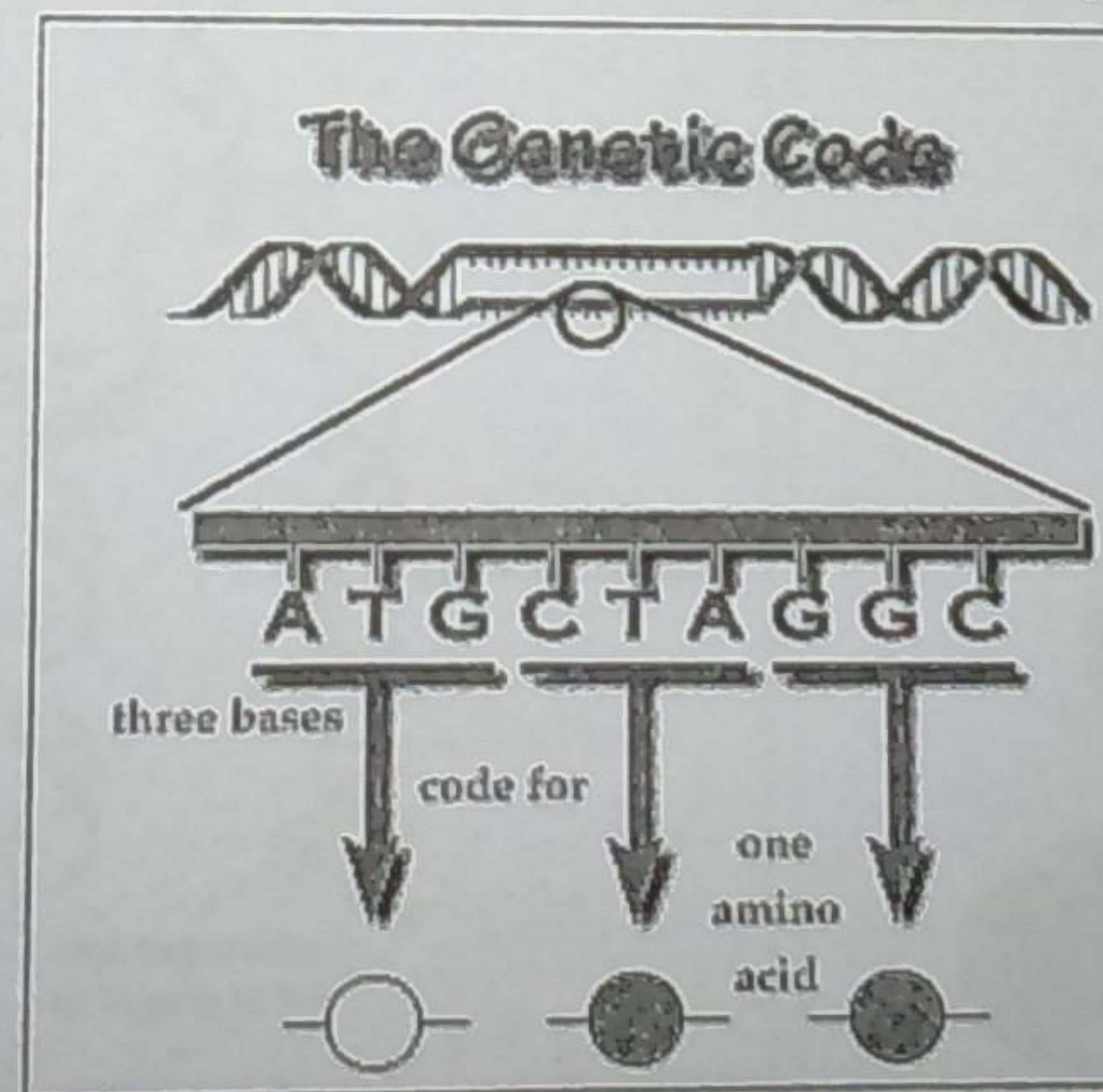
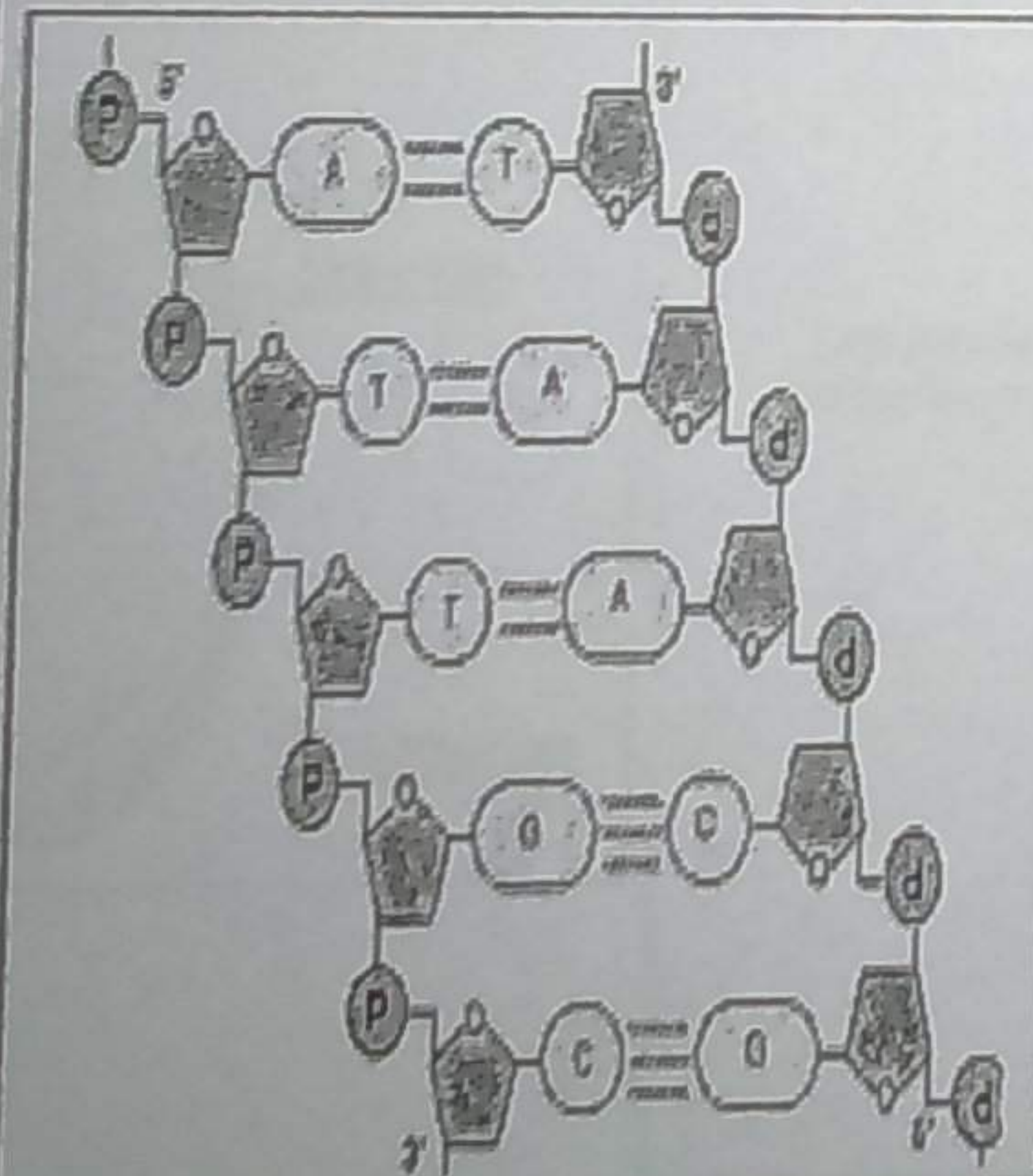
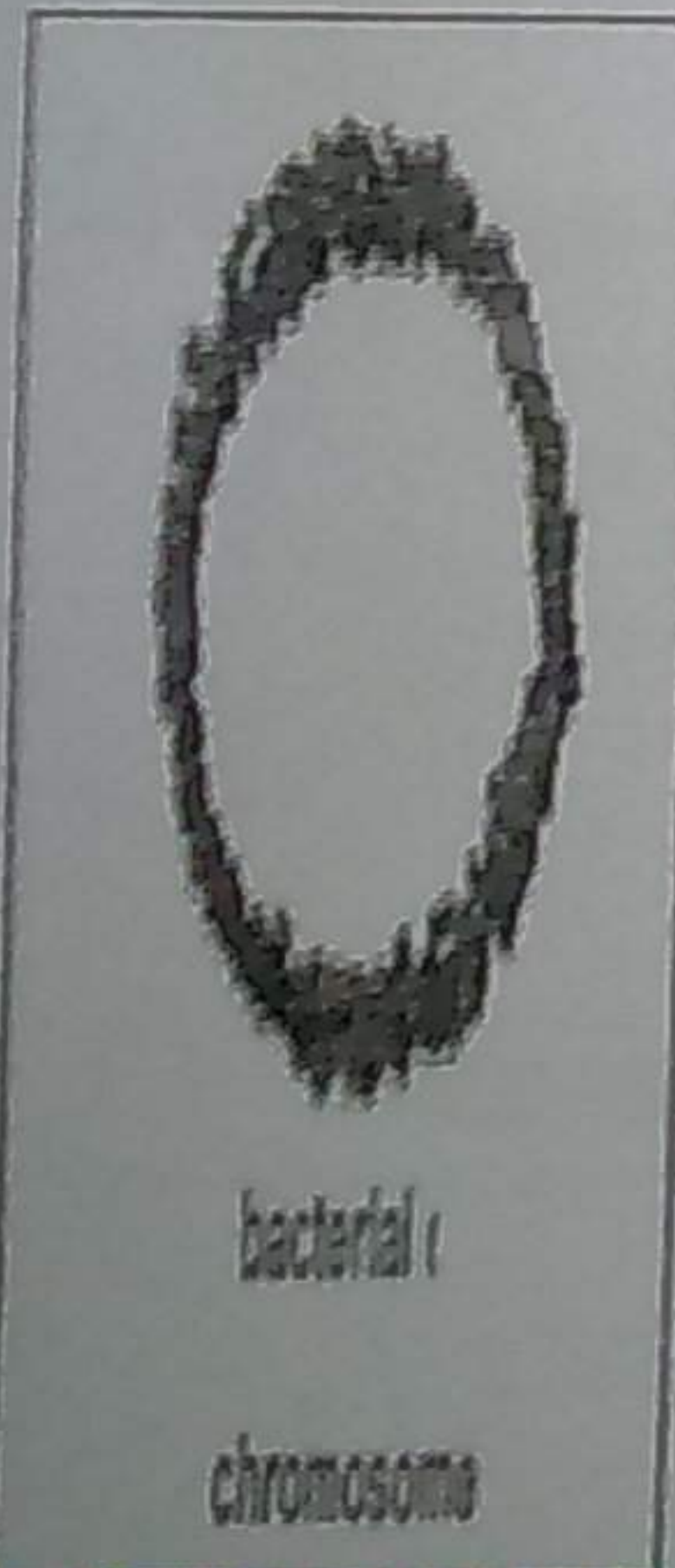
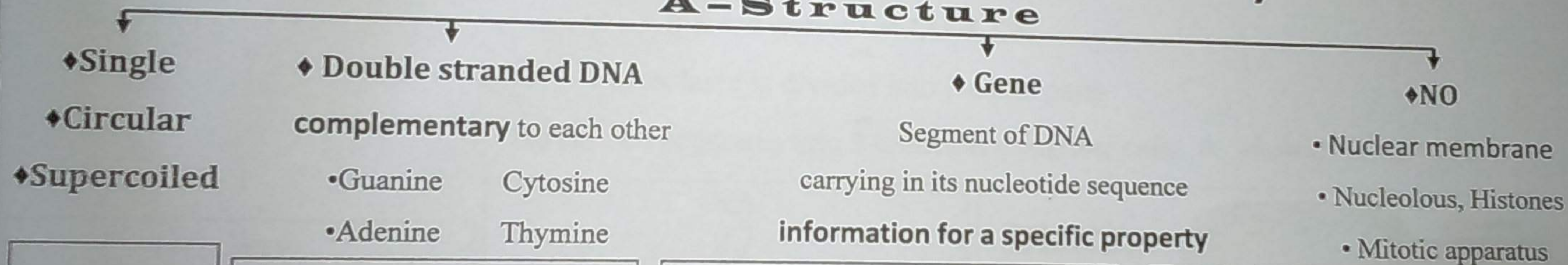
Bacterial Genetics

# Bacterial Genetics



## Bacterial chromosome (Nucleoid)

### A-Structure



## B-Functions

Carries essential genes controlling properties & pathogenicity

## C-Replication

Simple binary fission (semiconservative replication) *i.e. each daughter cell receive 50% of the original chromosomes*

The 2 strands separate

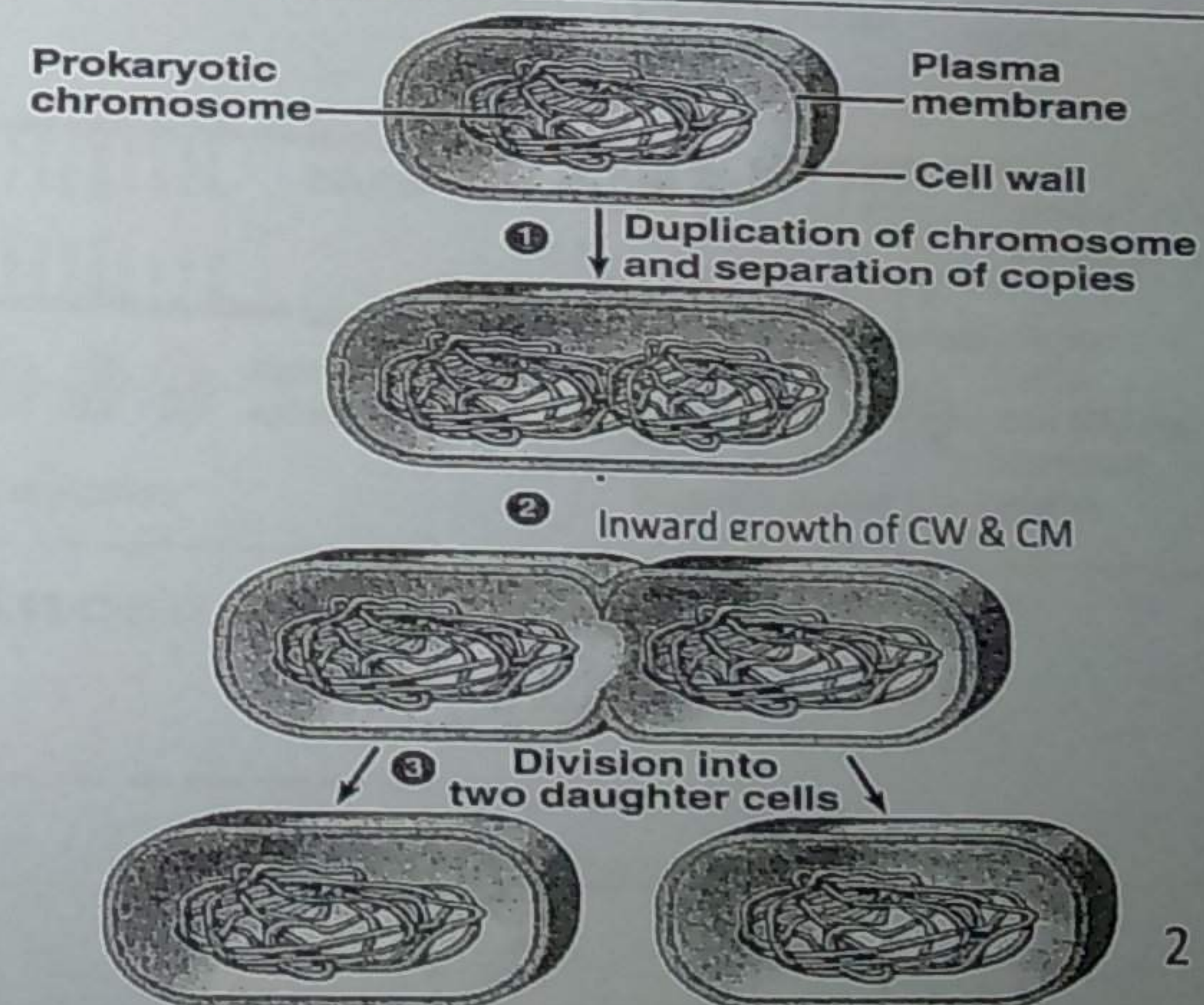
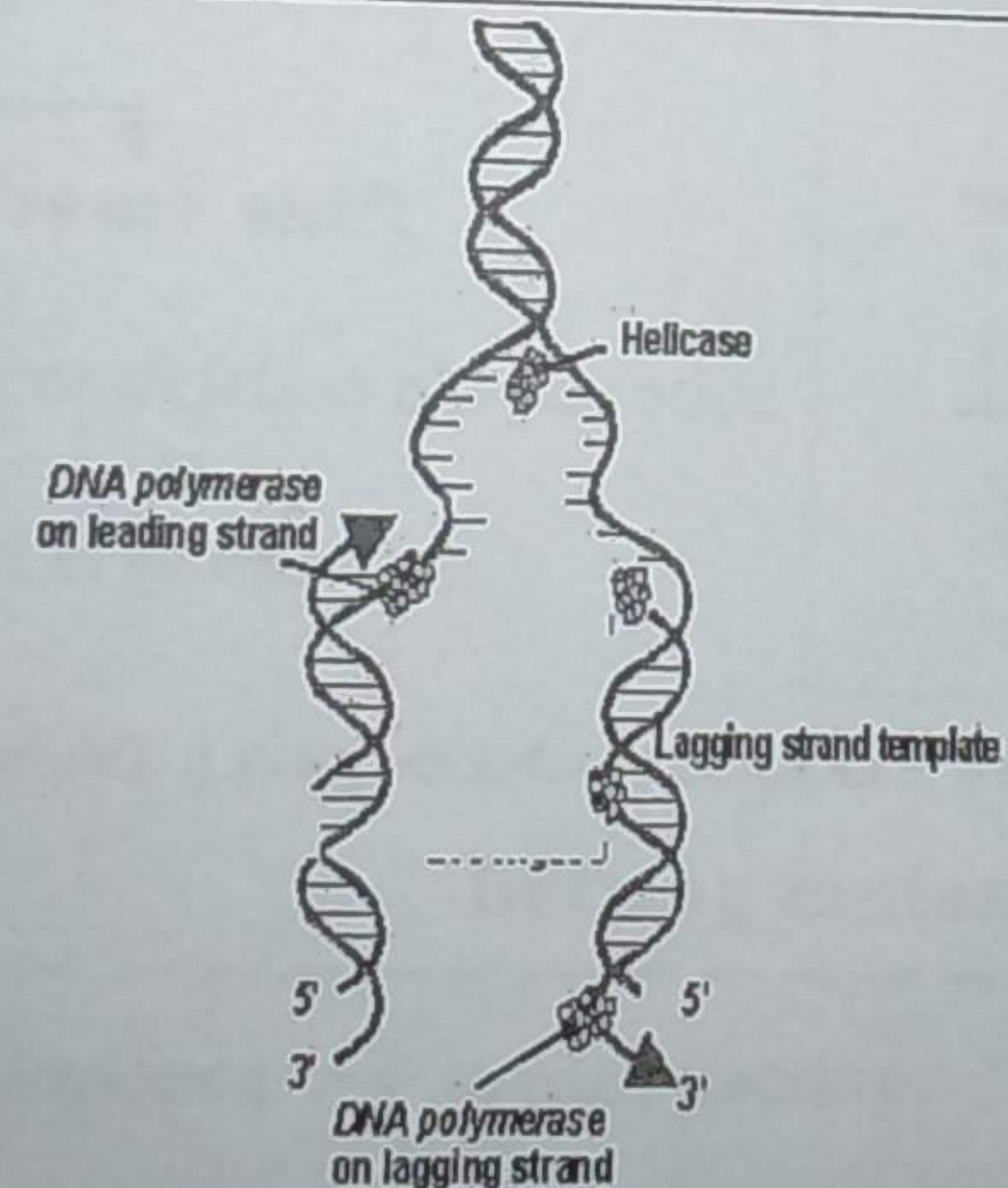
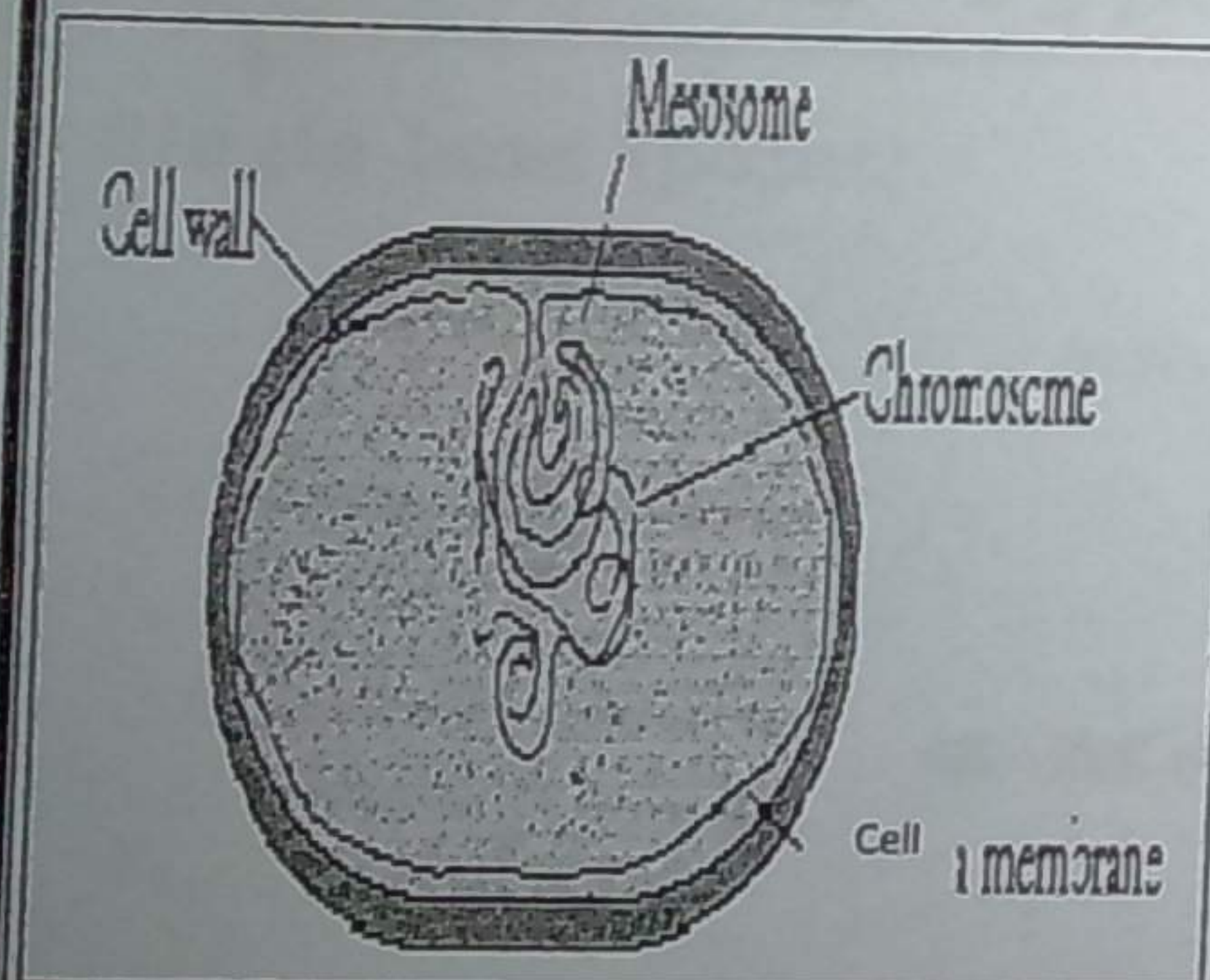
Each strand attaches itself to a **septal mesosome** & acts as a template

Synthesis of complementary strand by **polymerase**

Inward growth of **CM & CW**

Protoplasm is divided into 2 equal parts

Parent cell separates into **2** identical daughter cells & identical to the parent.



# Mutation

## 1 - Definition

Change in nucleotide sequence along DNA molecule

## 2 - Origin

Spontaneous

Replication

error

Induced

Physical

• UV

• γ rays

Chemical

♦ Alkylating substances

♦ Nitroso substances

## 3 - Types

Single base (point)

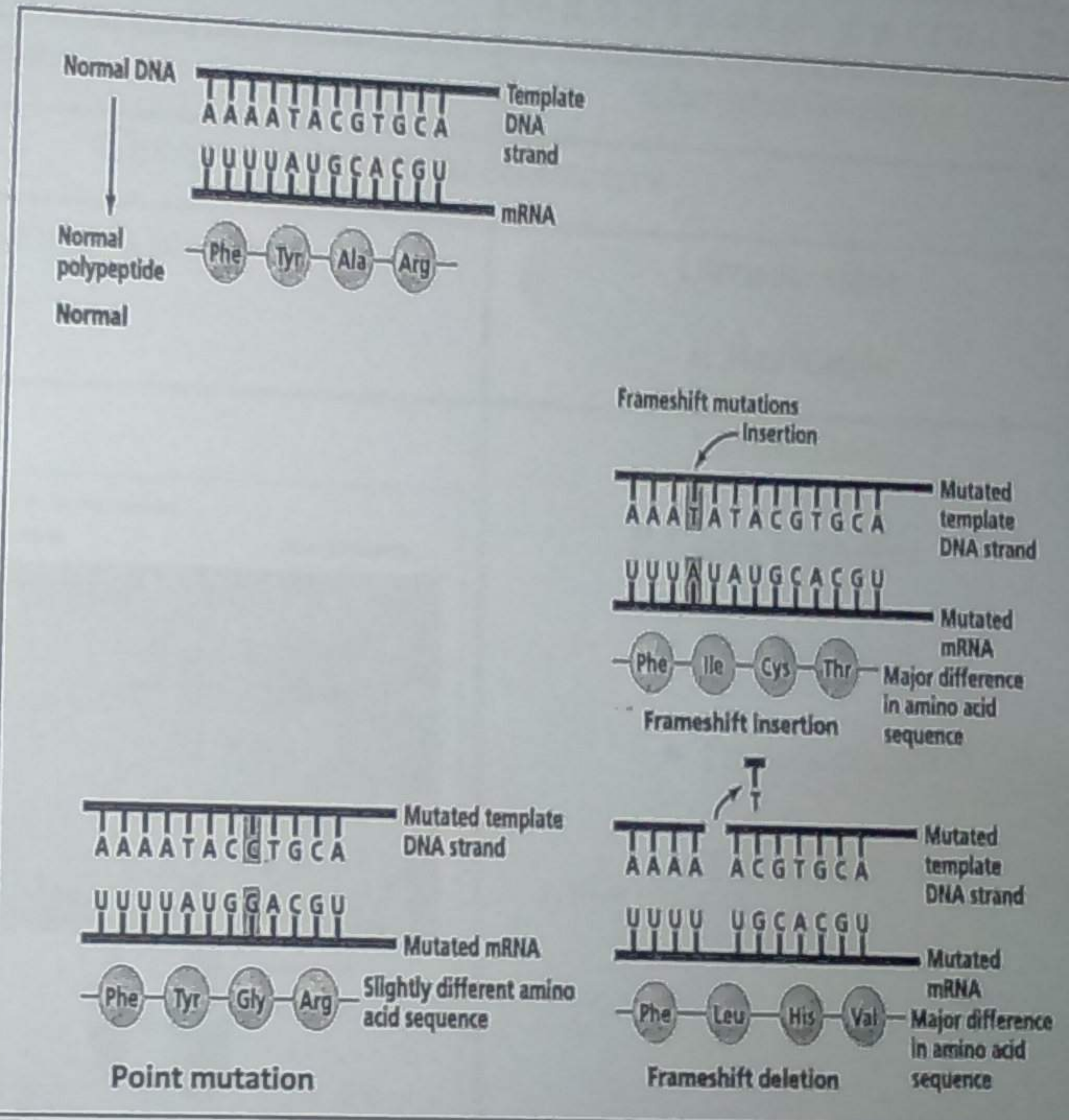
Replacement

of one nucleotide

Frame shift

Insertion or deletion of nucleotide

Shift in genetic code



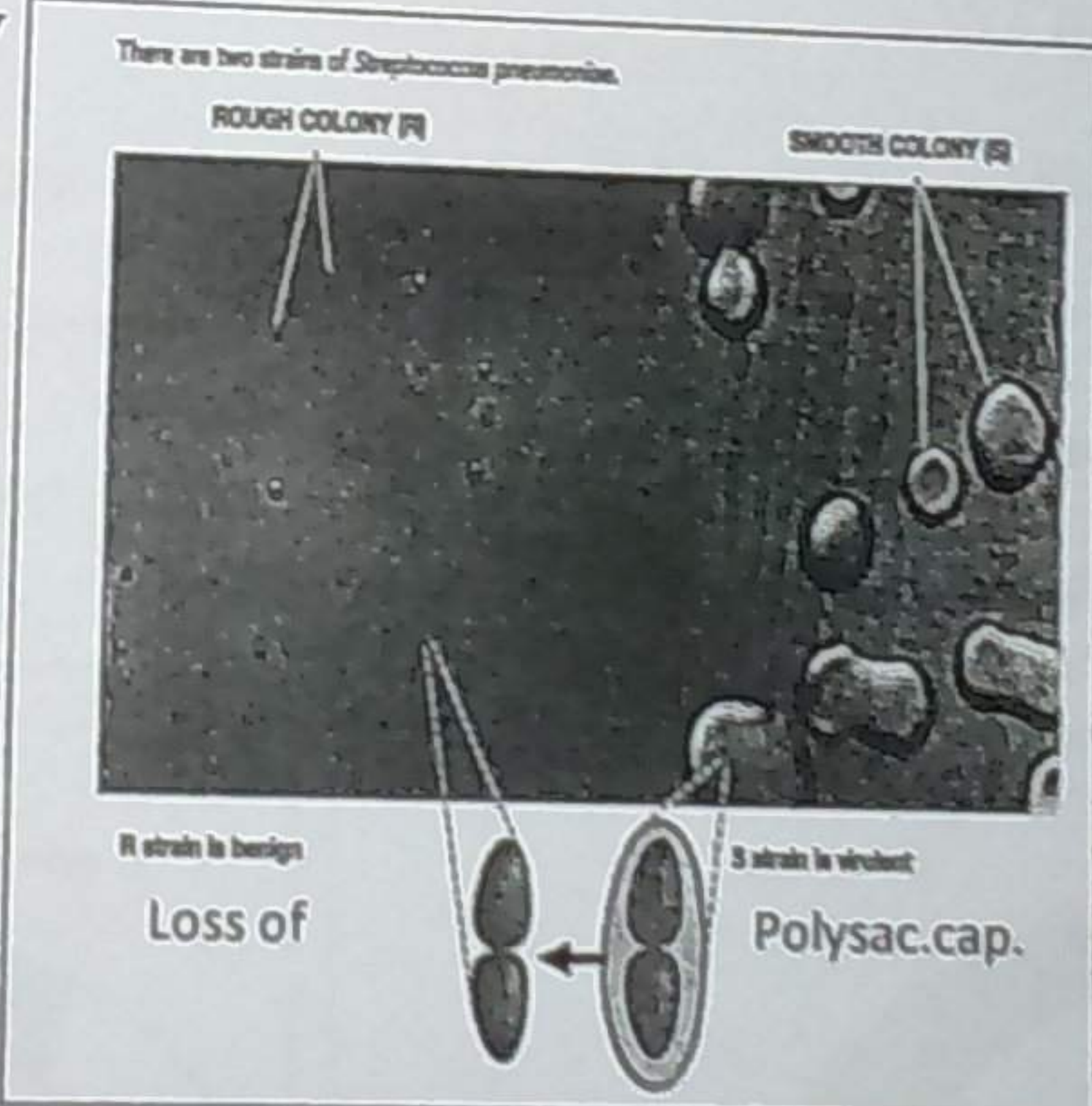
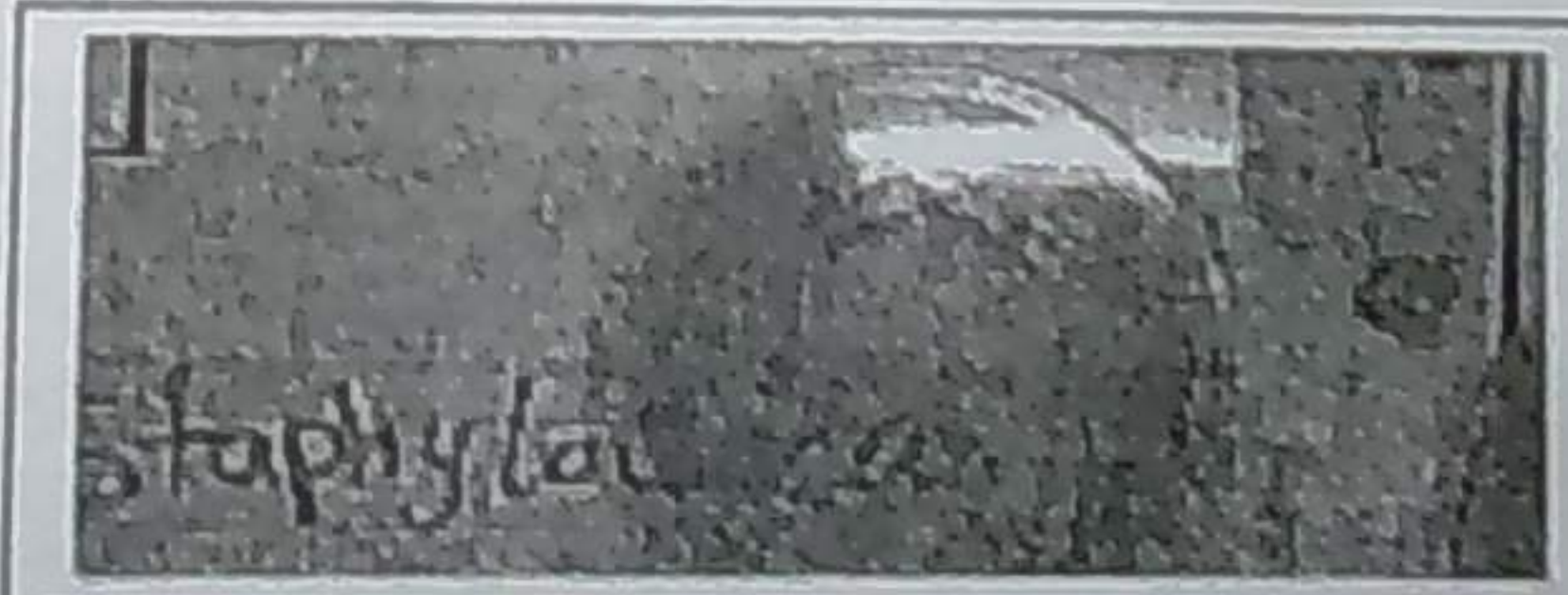
## 4 - Medical importance of induced mutation

Getting mutant

Of low virulence → used as vaccine

Producing large amount of antibiotics

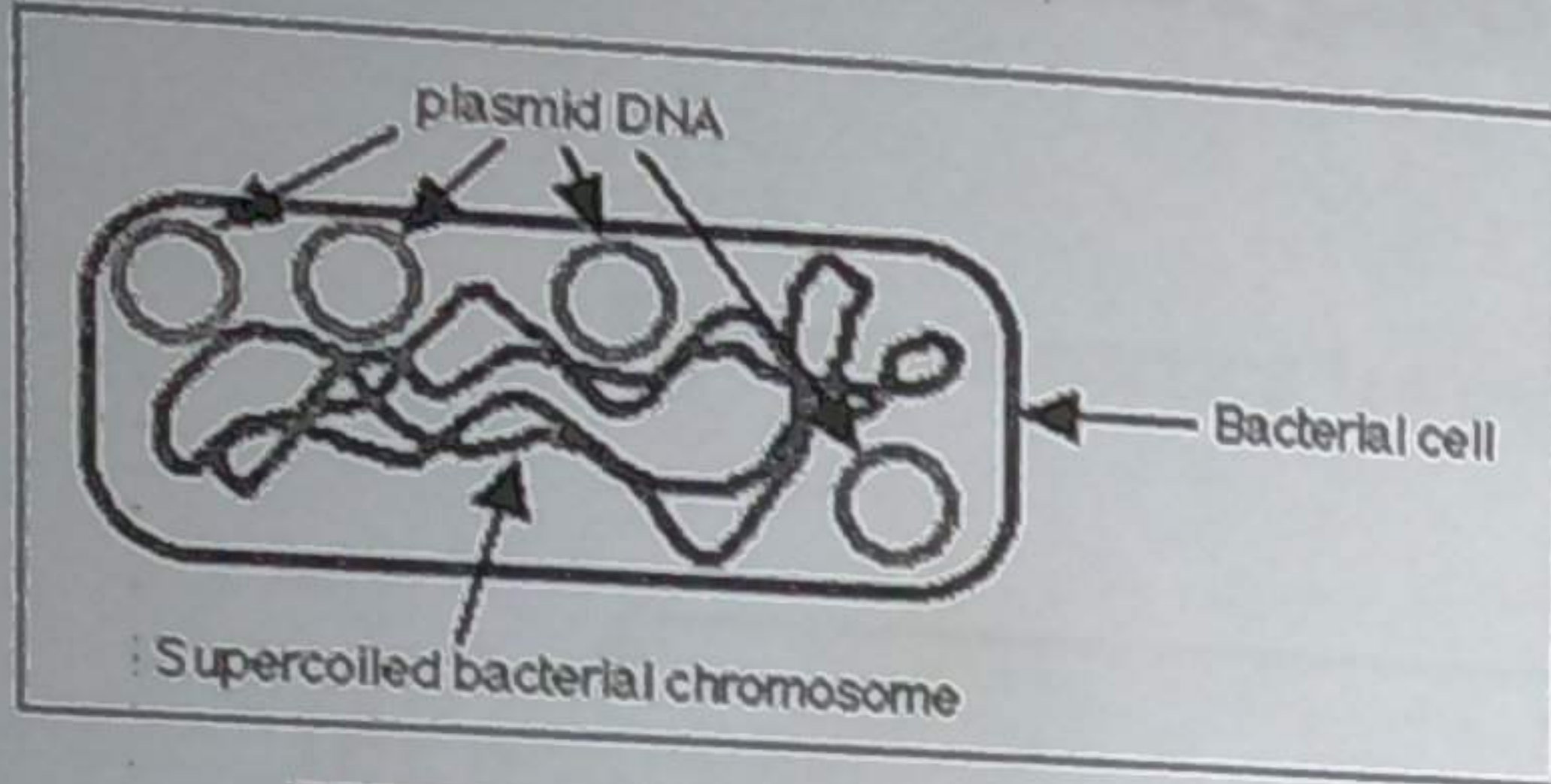
# Comparison between phenotypic & genotypic variation ★

	Phenotypic variation	Genotypic variation
1-Etiology	Environmental changes	Changes in genes
2-Effect	Changes in bacterial characters	
3-Characters	<p>i.Reversible : when environmental cause is removed</p> <p>ii.Non heritable</p>	<p>i.Irreversible</p> <p>ii.Heritable</p>
4-Examples	<p>i.Spore formation &amp; vegetation</p> <p>ii.Change in colonial morphology</p> <p>Bacterial growth on unsuitable environment  ↓  Smooth to rough (S-R) variation</p>  <p>iii.↑ endopigment production by Staphylococci</p> <p>If <i>milk</i> is added to the medium</p> 	<p>i.Mutation</p> <p>ii.Gene transfer :</p> <ul style="list-style-type: none"> <li>♦ Conjugation</li> <li>♦ Transformation</li> <li>♦ Transduction</li> </ul>

# Plasmids

## Structure

Circular Ds DNA: *Extrachromosomal & < the chromosome*  
↳ Double stranded.



## Characters & Properties

Dispensable

Not  
essential  
for  
bacterial life

Autonomously <sup>ذاتي</sup>  
replicate

Replicate independent  
of chromosome  
↳  
Many copies  
of the same plasmid  
may coexist in same cell

Recombination  
<sup>إحتزاج</sup>

Can  
integrate  
in chromosome

**Episome** ← MCQ

Transmissible

Transmitted  
to other

bacteria by:

- Conjugation
- Transformation
- Transduction

Self transfer (ST)

ST by conjugation

Plasmids of  
**G-ve** bacteria

carry **tra** genes

coding for **sex pilus**

No ST by conjug.

Plasmids of  
**G +ve** bact.

has no  
tra genes

Recomb.

Uninserted  
"plasmid"

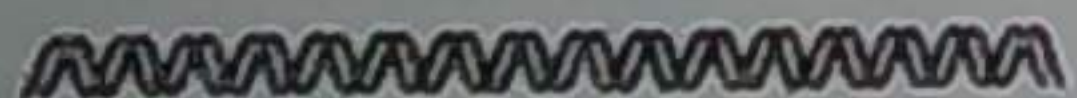


X



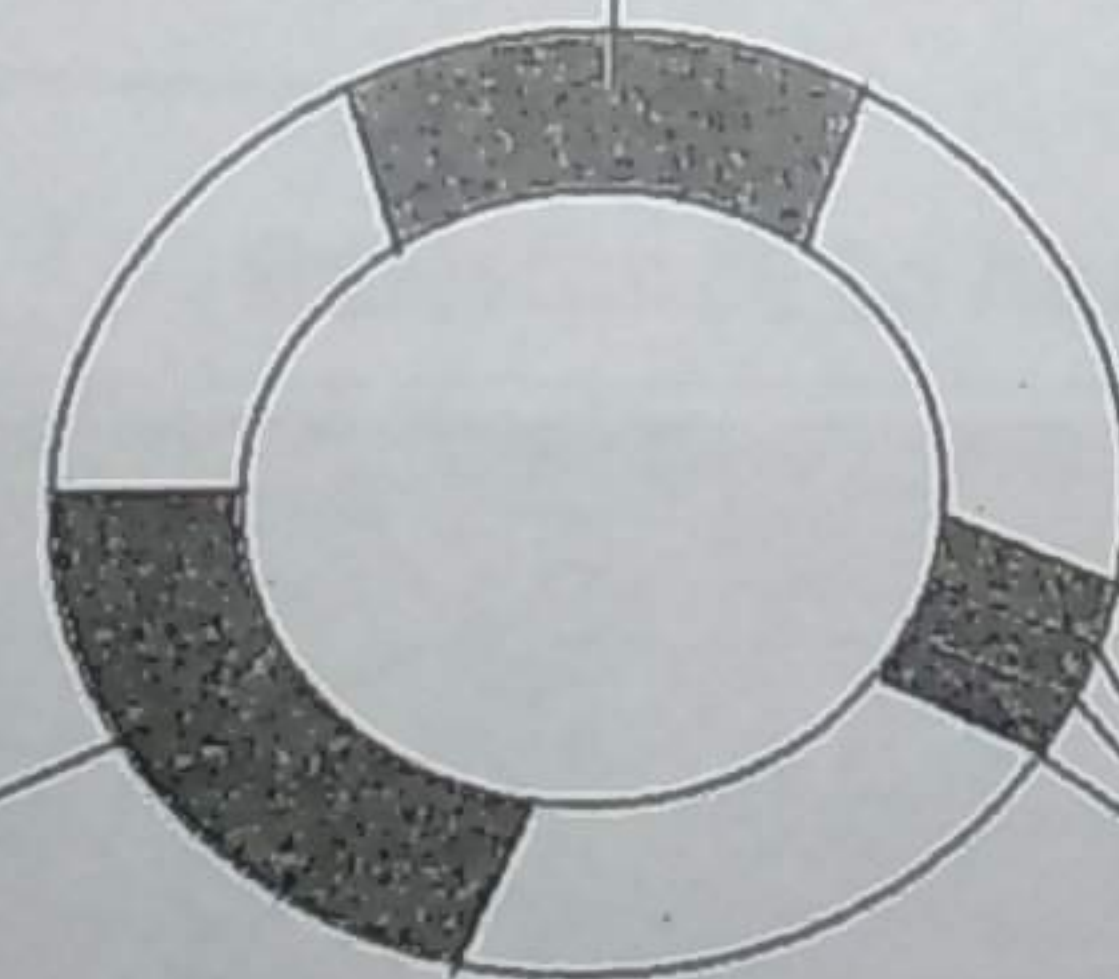
Bacterial chromosome

Episome



R plasmid of G+ve bacteria

Origin of replication



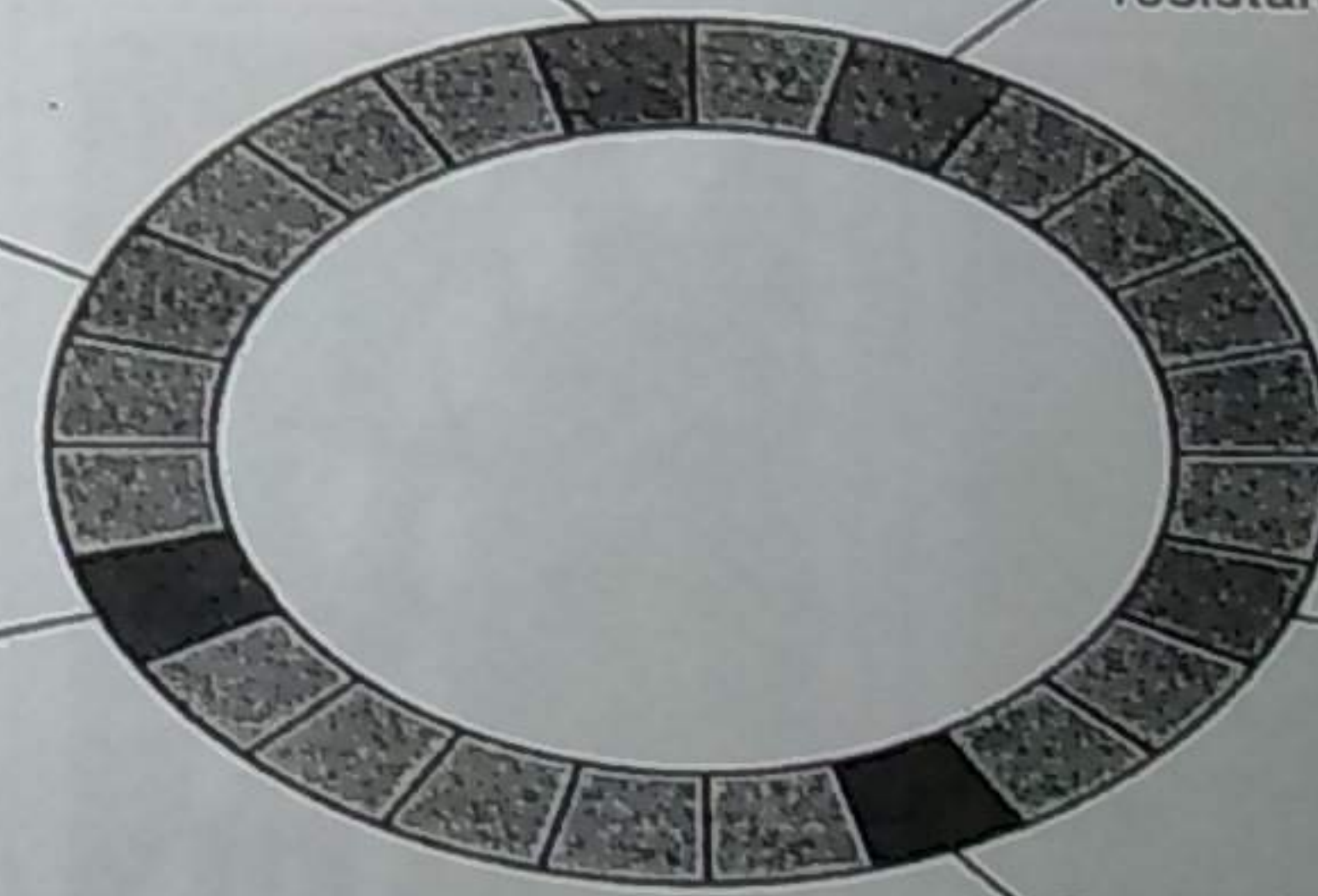
antibiotic  
resistance

PLASMID

R plasmid of G-ve bacteria

Antibiotic  
resistance gene

Origin of  
replication



Tra  
gene

Origin of transfer

R genes

# Cell properties determined by plasmids

## 1-Sex pilus formation

F plasmid carries tra genes → sex pilus → gene transfer by conjugation (only in G<sup>-ve</sup>)

↳ No Tra gene in any plasmid of G<sup>+</sup>ve

## 2-Virulence plasmids TARI

Tox plasmids

e.g coding for *enterotoxin*  
in E.coli

Adhesion plasmids

e.g coding for *pili*  
in E.coli

Resistance to antibiotics: R plasmid

e.g coding for  $\beta$  lactamase  
that degrades penicillins

Invasion plasmids

e.g in Yersinia

↳ Coding for invasin protein  
for VF

## 3-Production of anti-bacterial substances

Bacteriocin (colicin) production : e.g Col plasmid of E.coli

Antibiotic-like substance produced by certain bacteria  
to kill other bacteria of same or closely related species

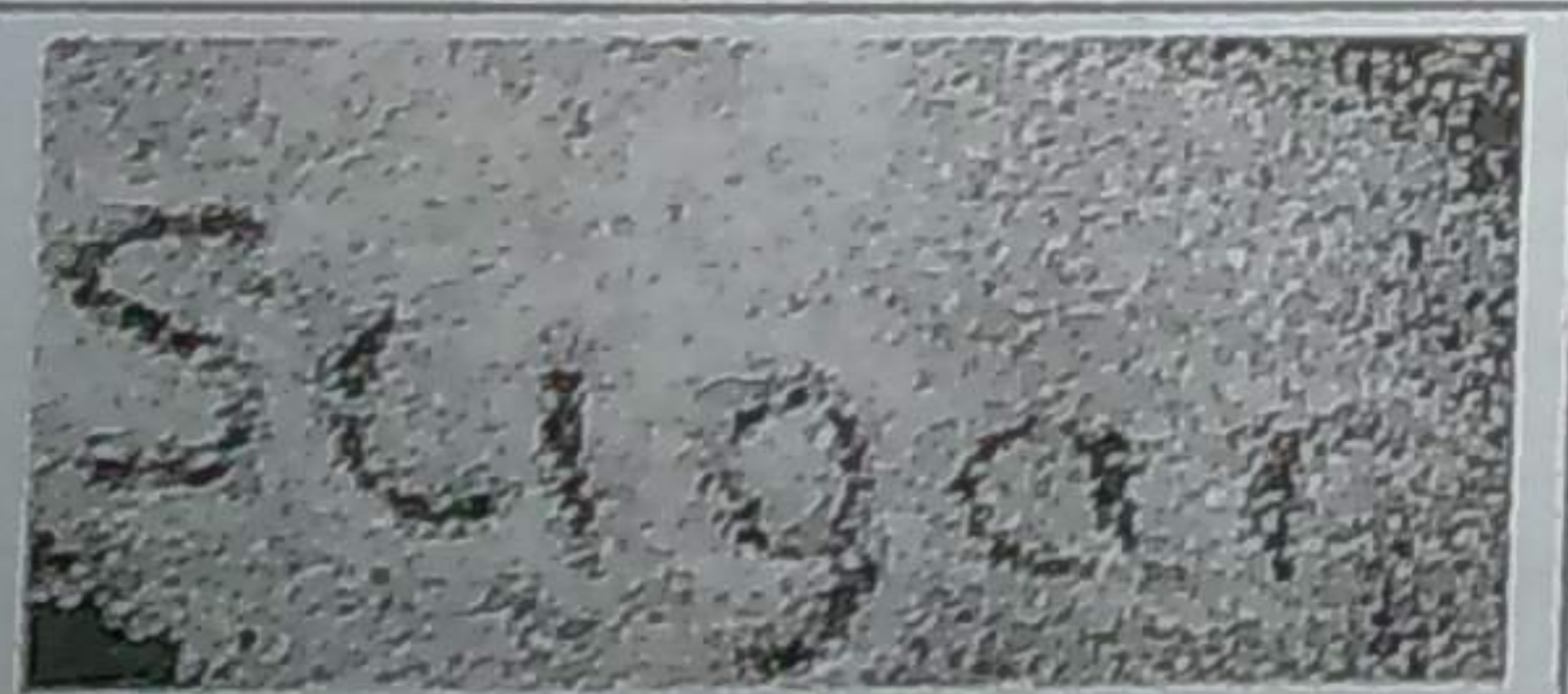
Antibiotic production

↳ It kills related & unrelated species -

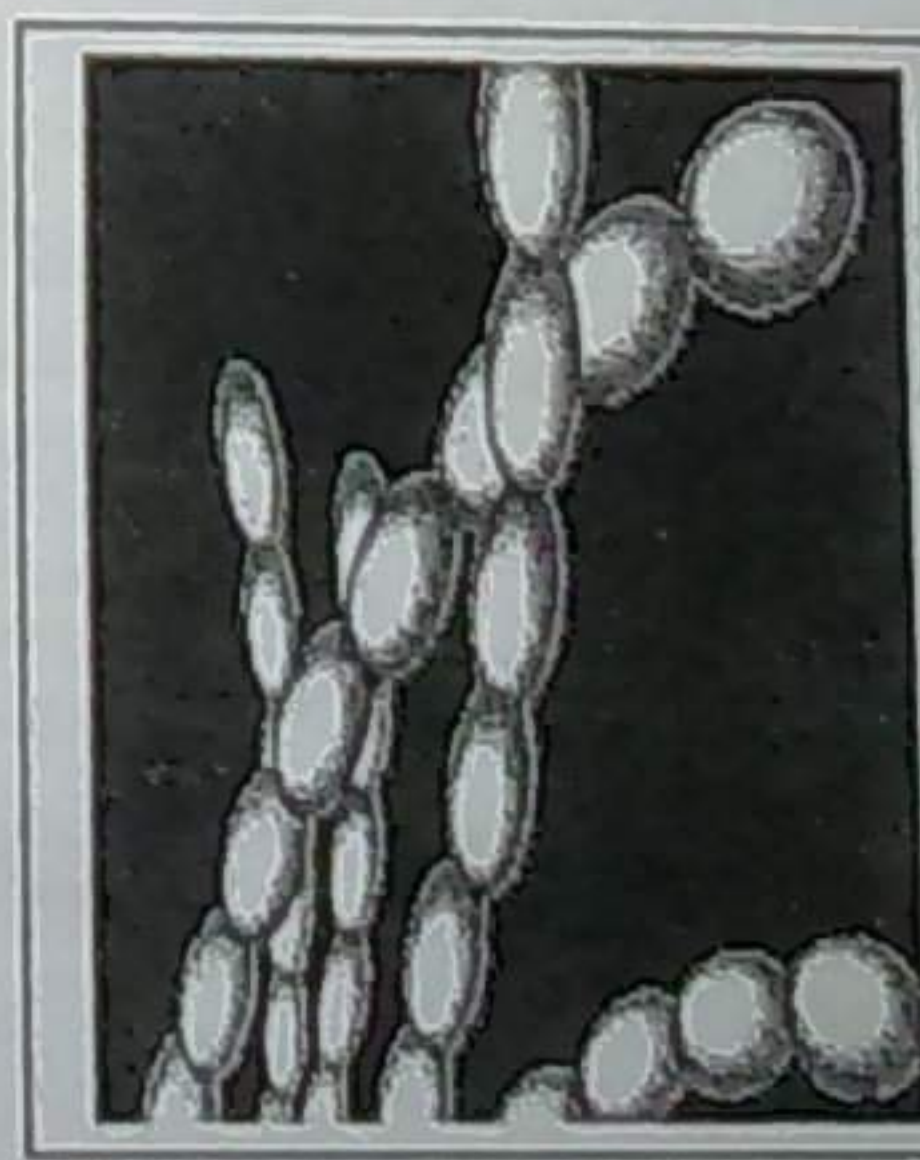
e.g Plasmid in *Streptomyces*  
coding for *streptomycin*

## 4-Biochemical activities (In Vitro)

Sugar fermentation



Resistance to heavy metals



# Conjugation : gene transfer by sex pilus ( only in G-ve )

Transfer of F plasmid

(Fertility or sex plasmid)

Sex pilus of donor  $F^+$  cell

comes in contact with recipient  $F^-$  cell

Endonuclease cleaves 1 strand  
of F plasmid at origin of transfer

Unwinding of 2 strands of F plasmid  
by helicase enzyme

1 strand passes through sex pilus to  $F^-$  cell

Complementary strand is formed by both cells  
by *polymerase*

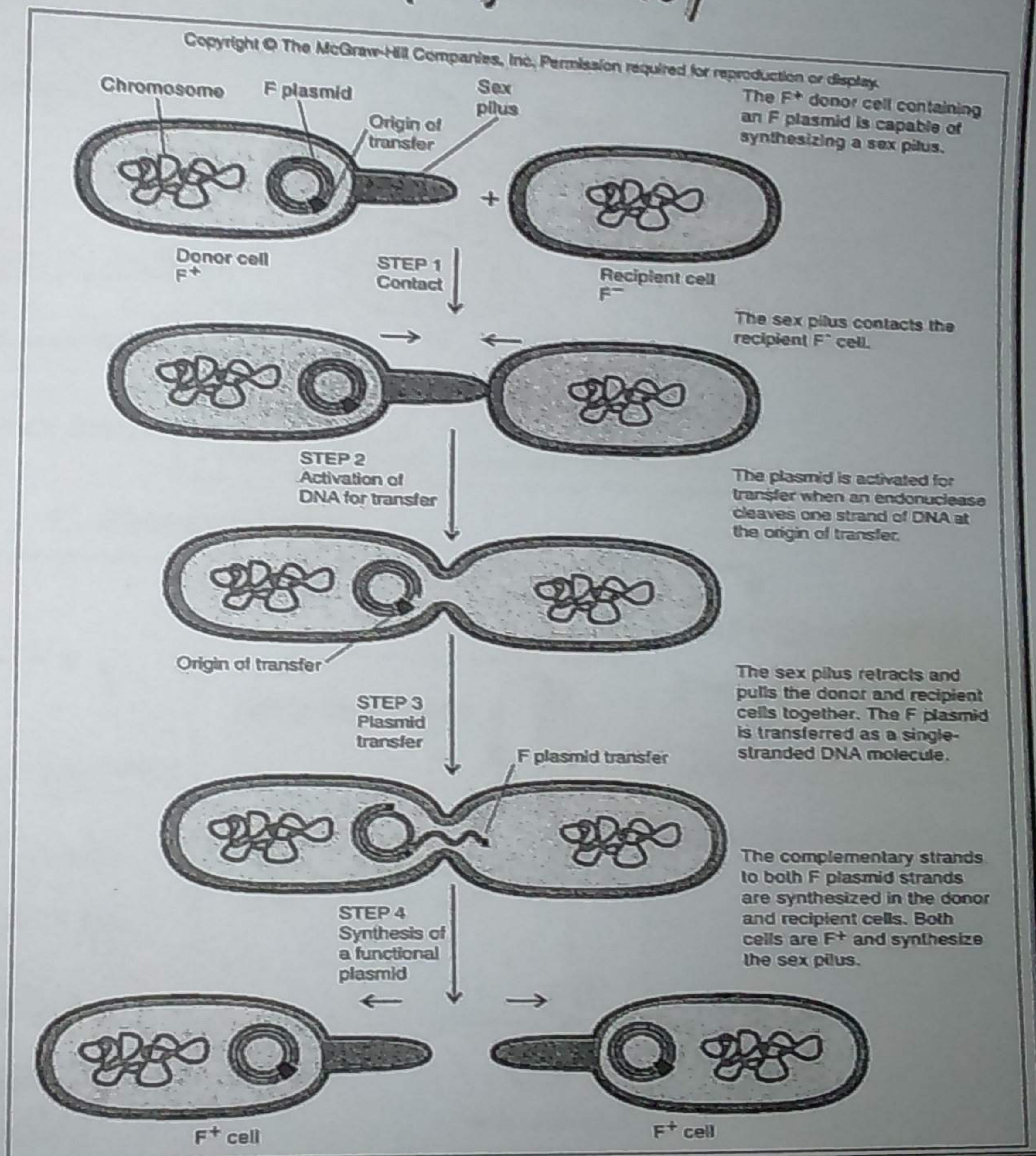
$F^-$  recipient cell is changed to  $F^+$  cell

NB

Plasmid coding for penicillinase in Staph.(G+ve)

can't be transferred by conjugation

Transferred by transduction



# Transformation

## A-Definition

Uptake of **free naked DNA** ( part of chromosome or plasmid )

## B-Mechanism

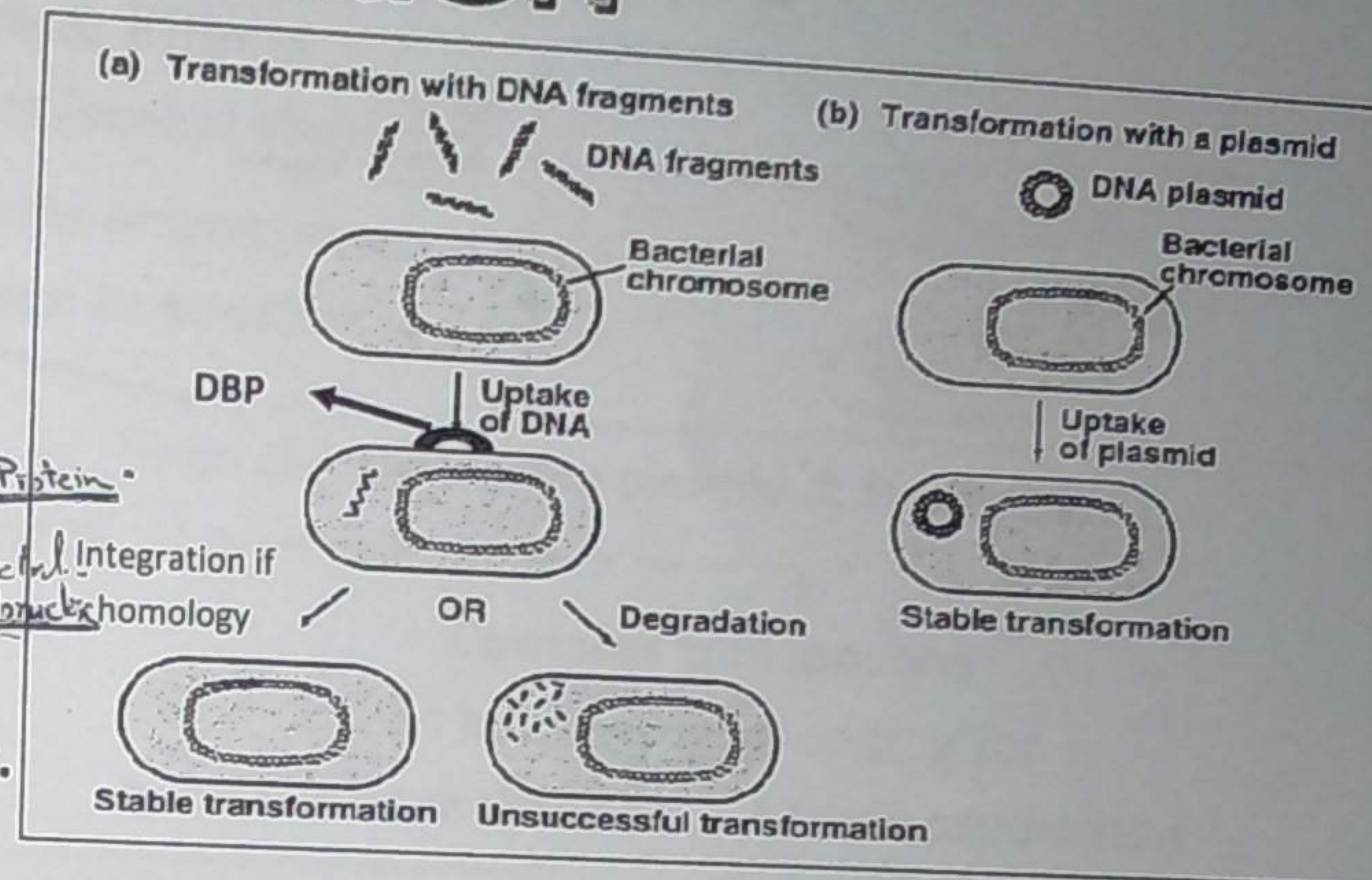
Dead bacteria release DNA

↓  
Binds to **competent** recipient bacterium

↓  
Recombines with its DNA ( or remains free if it is plasmid )  
↓  
New character if DNA carries new gene e.g **virulence factor**.

DNA-Binding Protein

Weak or absent restriction endonuclease activity



## C-Prerequisites

### Competence

**Natural competence** is uncommon

due to presence of

**restriction endonuclease**

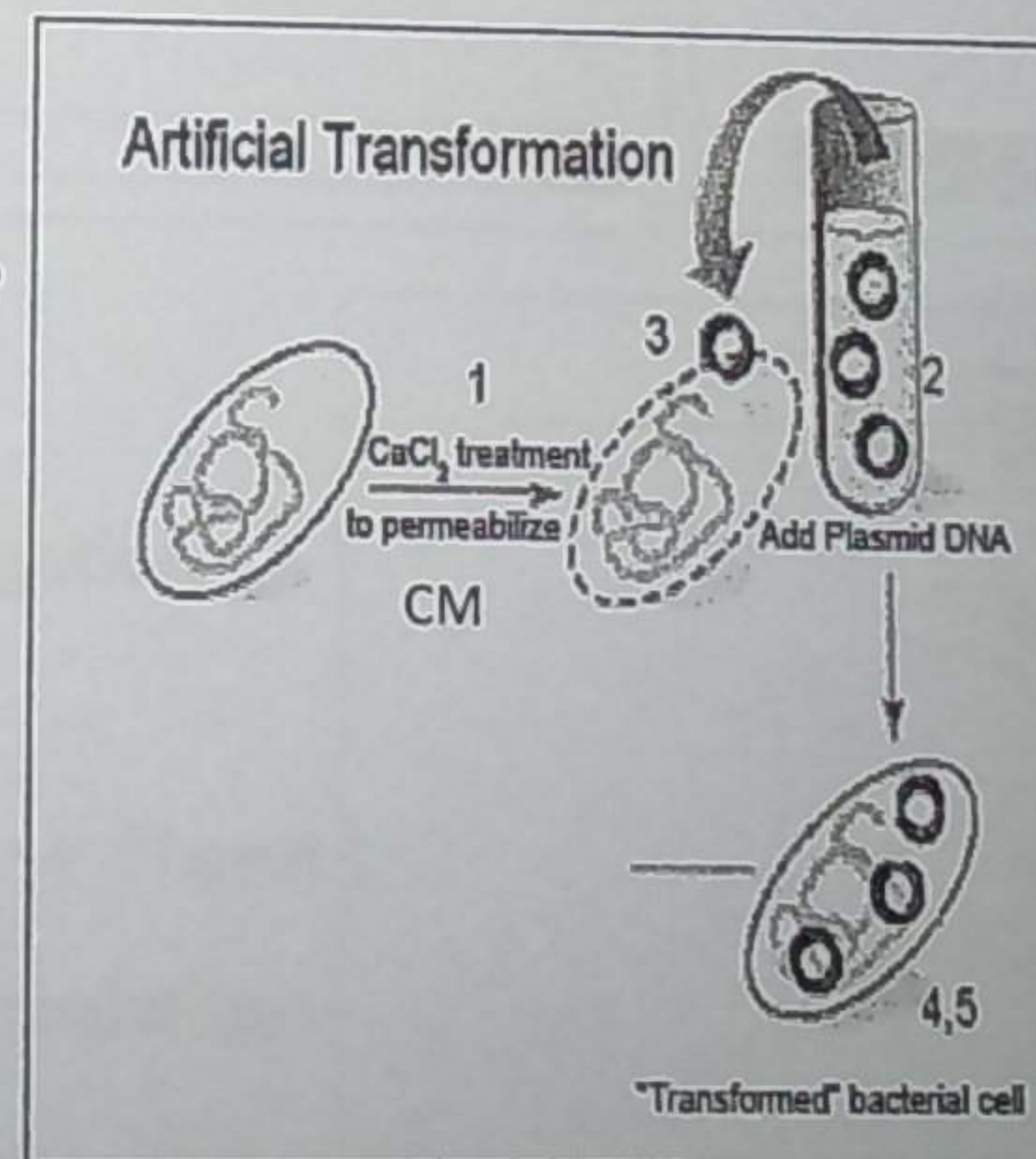
which digests

foreign DNA

**Artificial competence**

Treating cell with  
**CaCl<sub>2</sub>** or heat shock

↓  
↑ CM permeability  
( Used in **genetic engineering** )

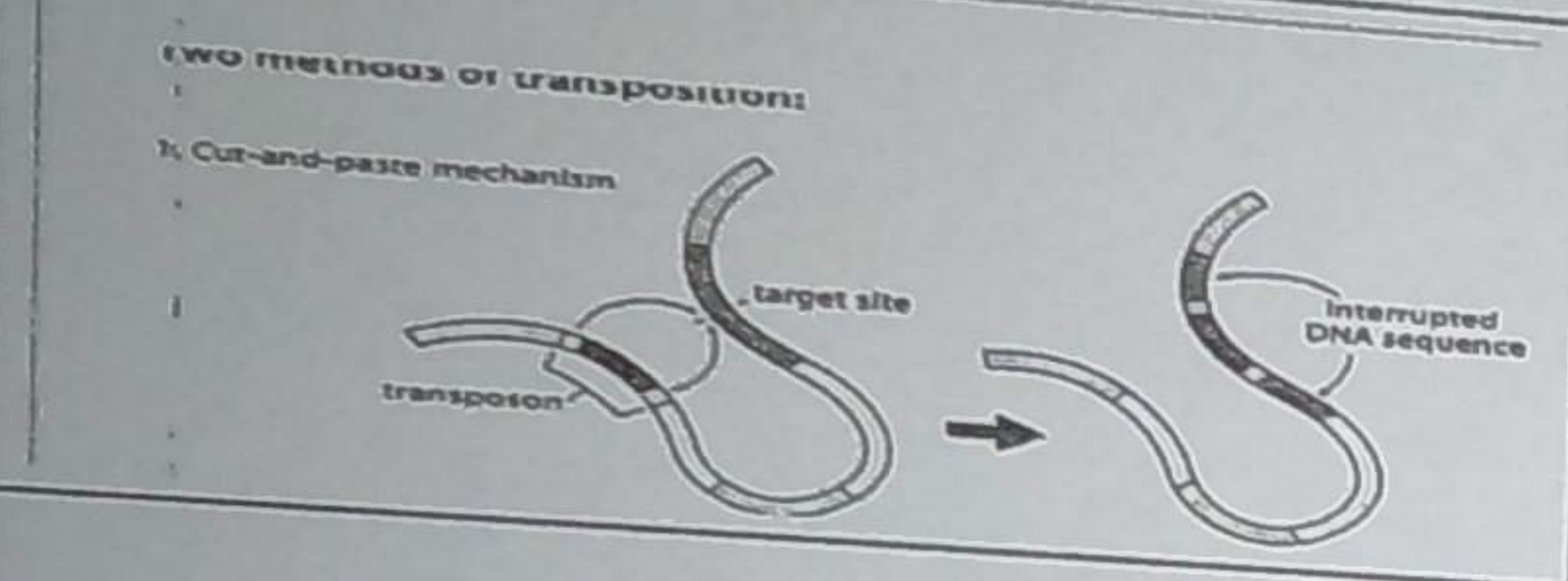


Homology between  
donor & recipient

Absence of homology  
prevents recombination

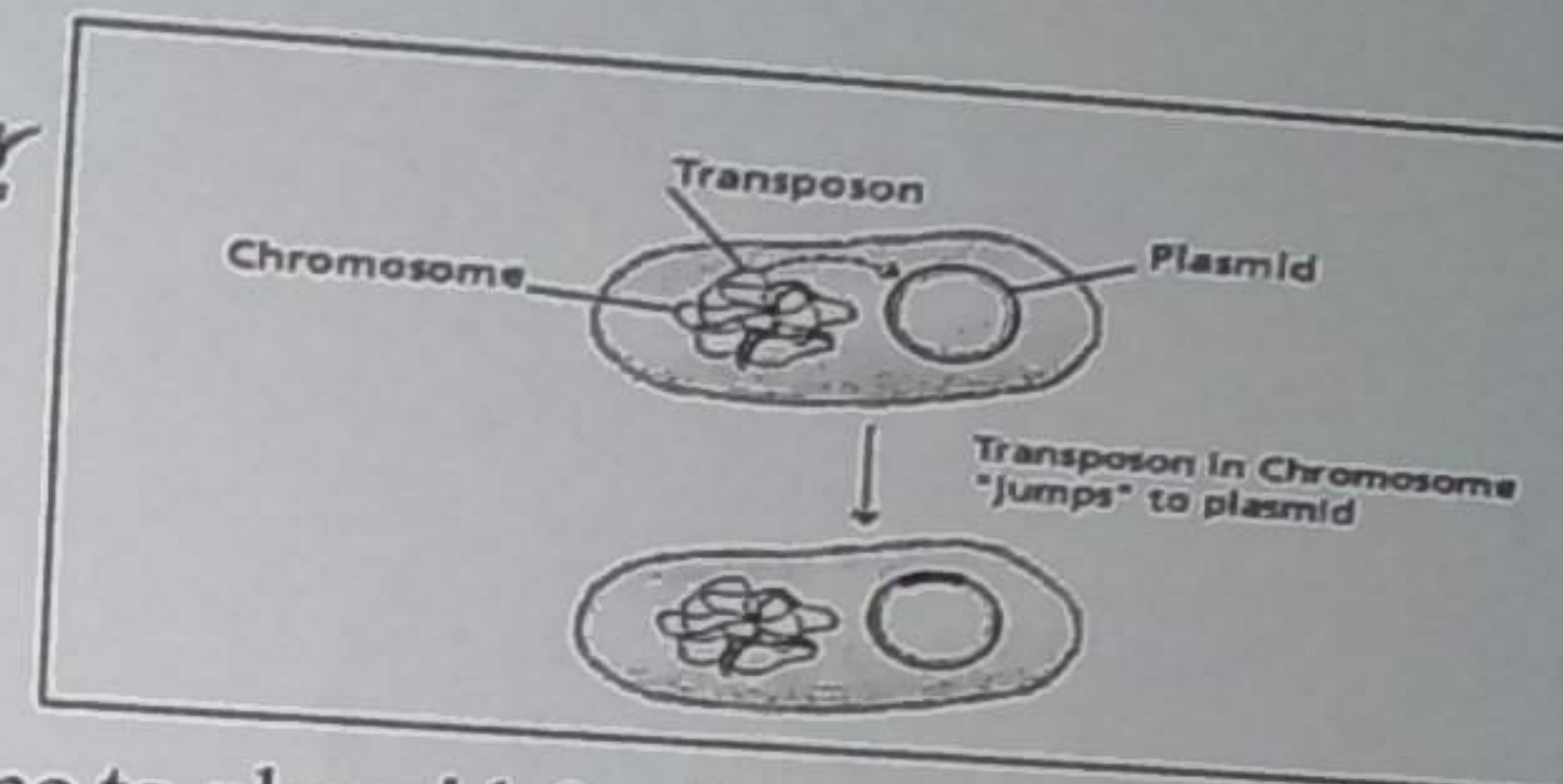
↓  
DNA is degraded

# Transposons : jumping genes



## A-Structure & Effects

Genetic elements (several kbp) <sup>kilo base pairs</sup> that can **move** (jump or transpose) from one location to another

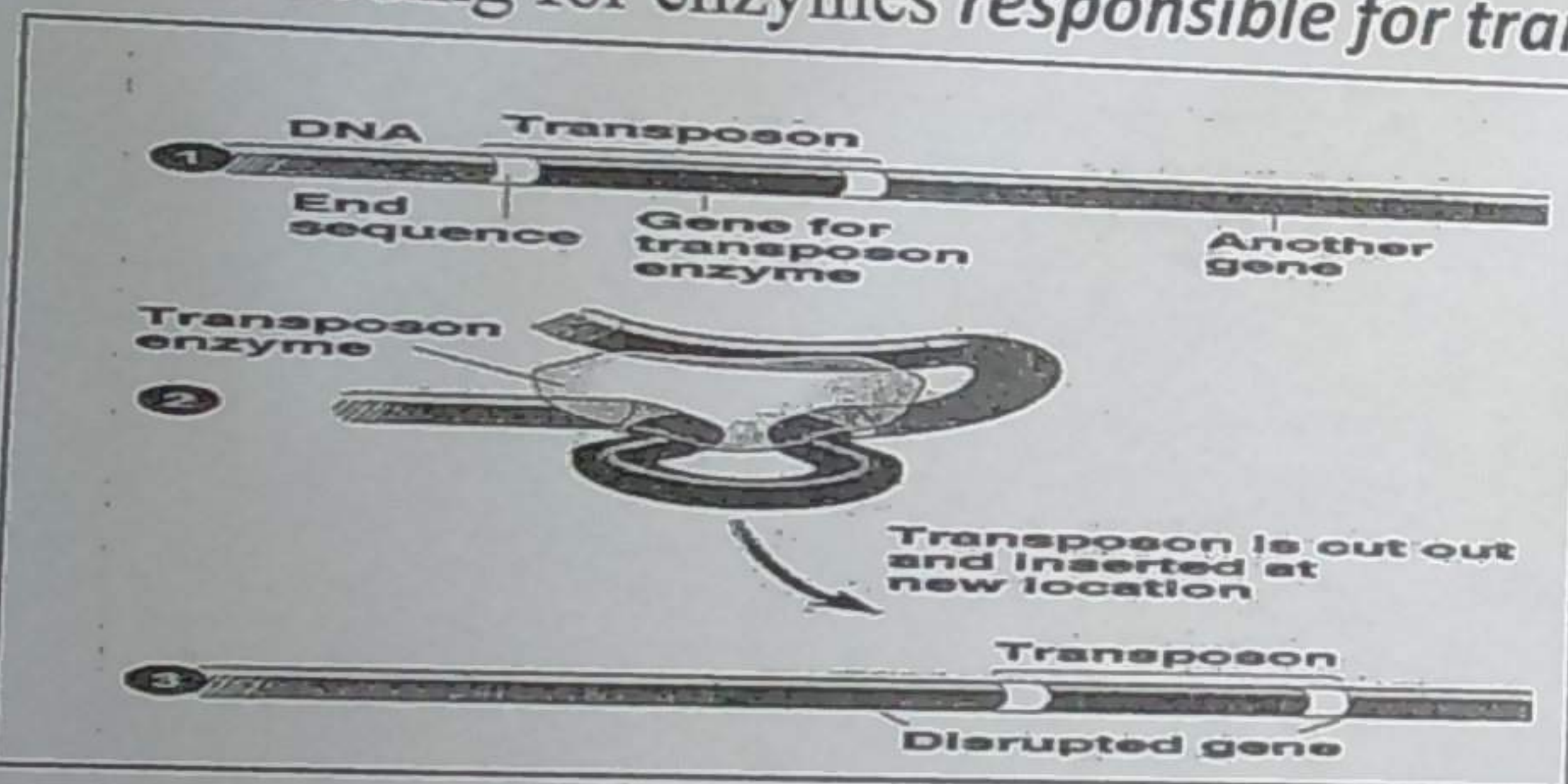


On the same chromosome

From chromosome to plasmid & vice versa

## B-Types

Simple insertion sequence  
Genes coding for enzymes *responsible for transposition*

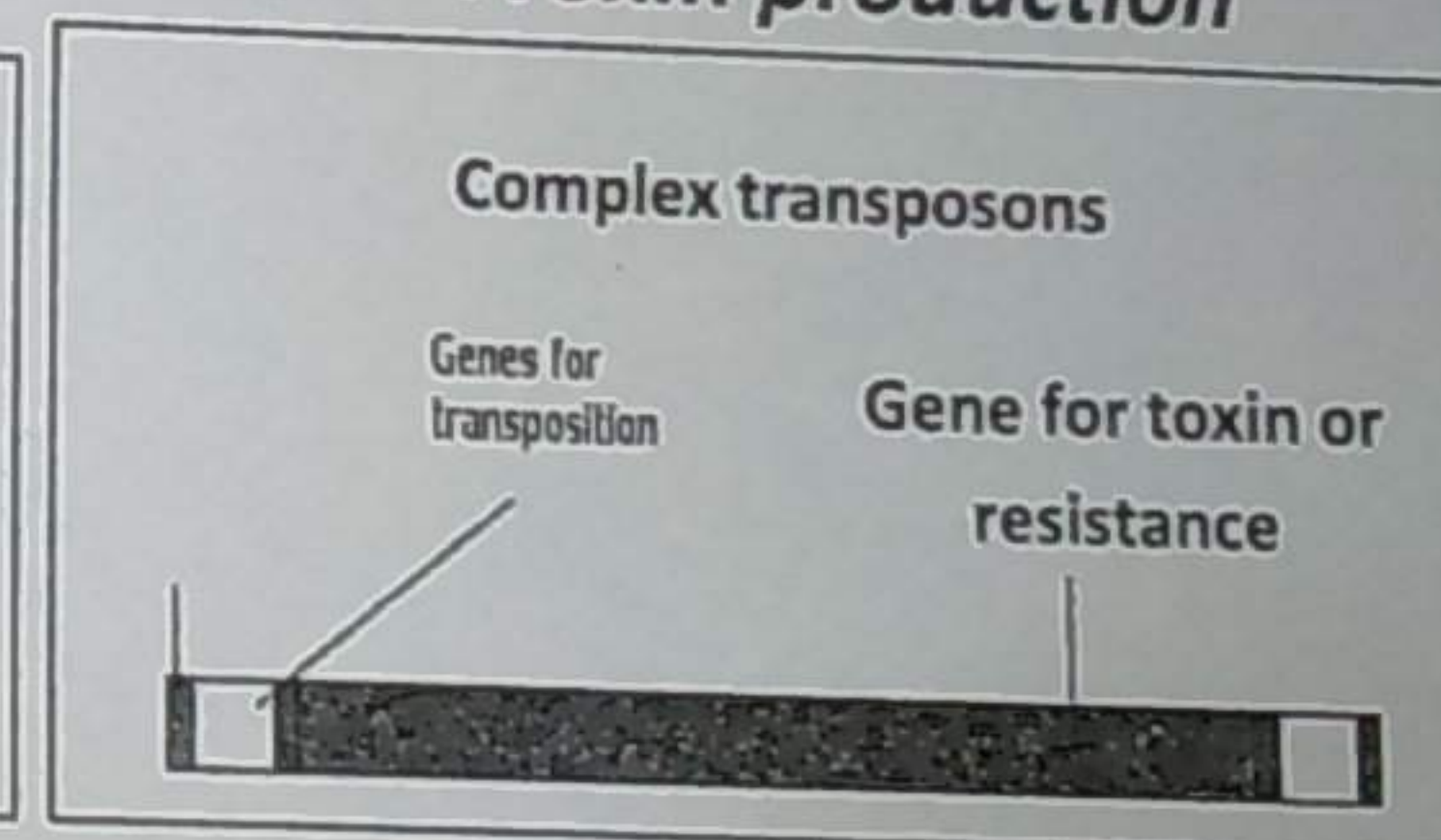
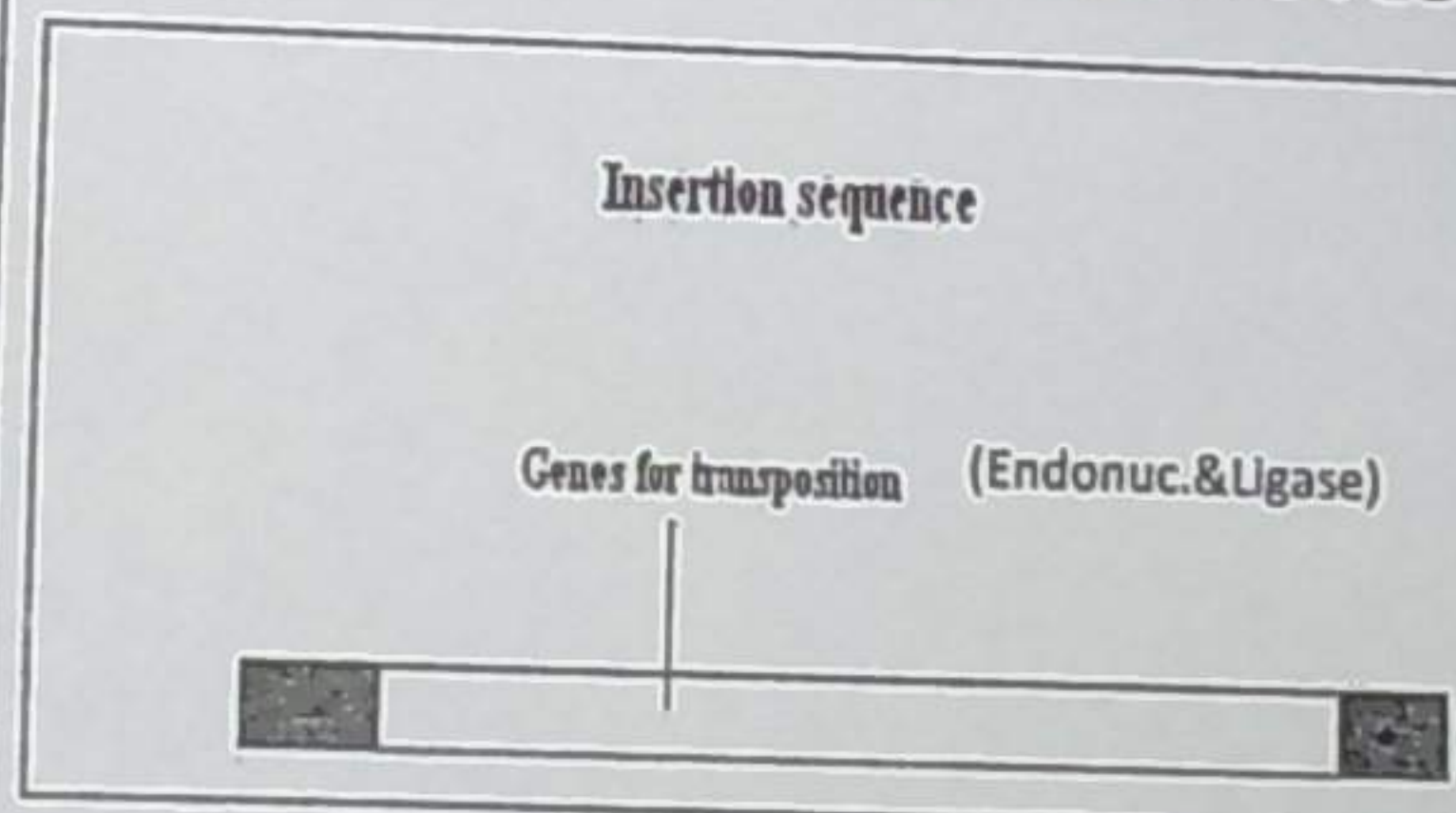


## Complex transposons

As before + gene coding for

♦ Antibiotic resistance

♦ Toxin production



## C- Characters

Can't autonomously replicate

Must replicate within a *replicon*

(self replicating DNA)

Chromosome

plasmid

NB: Transposons isn't ~~in cytoplasm~~

in cytoplasm

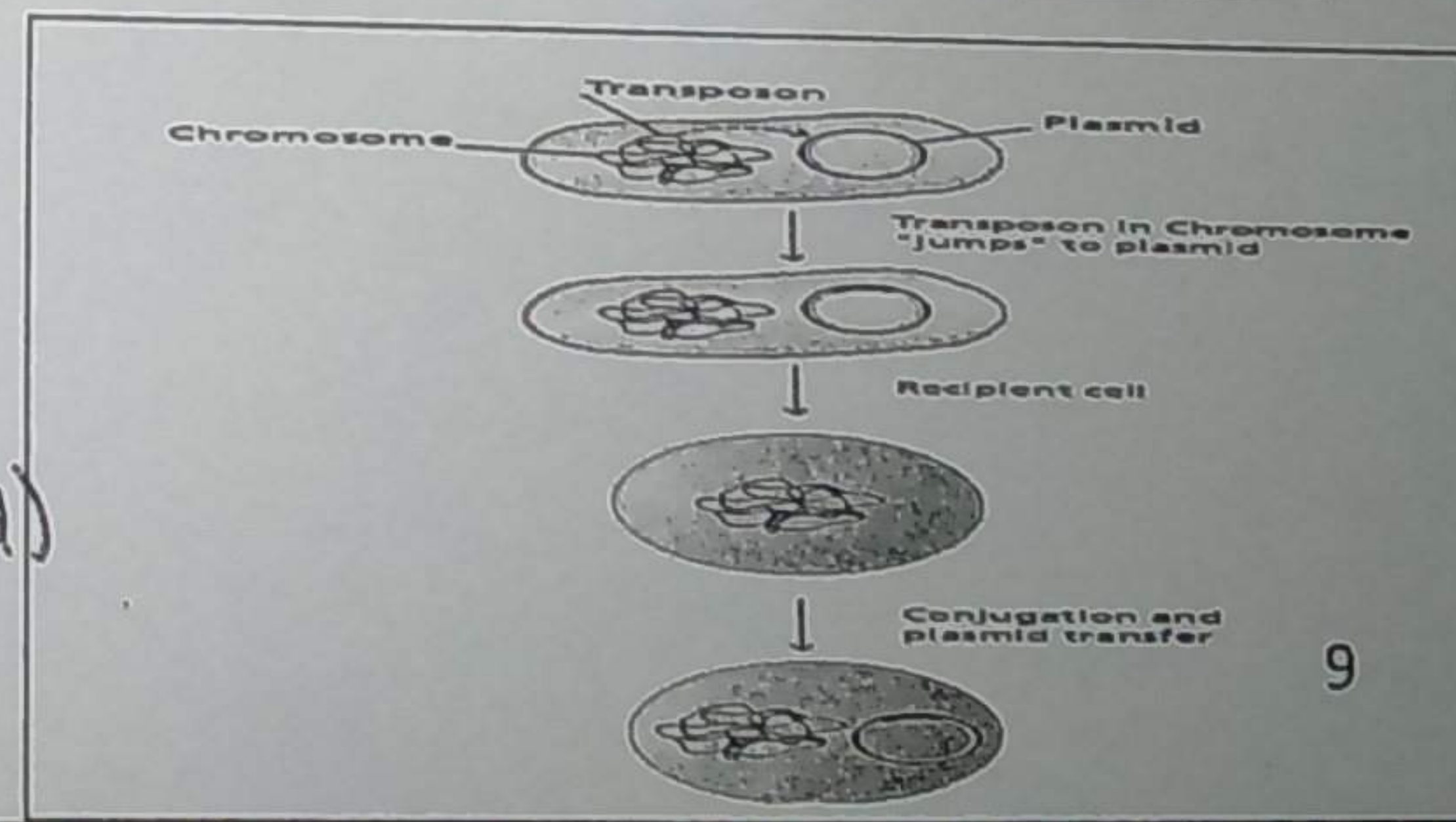
replicone

Transmissible : by conjugation

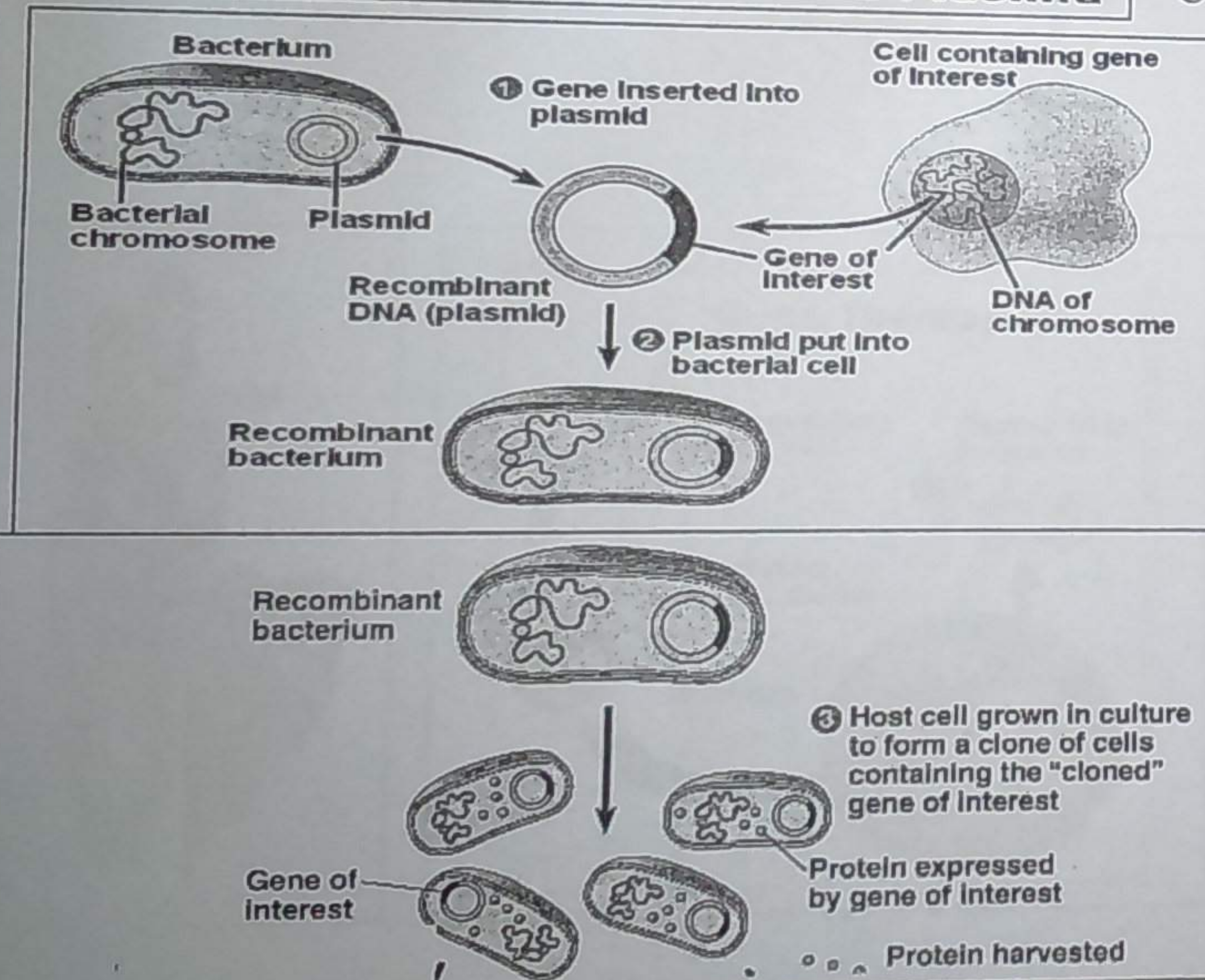
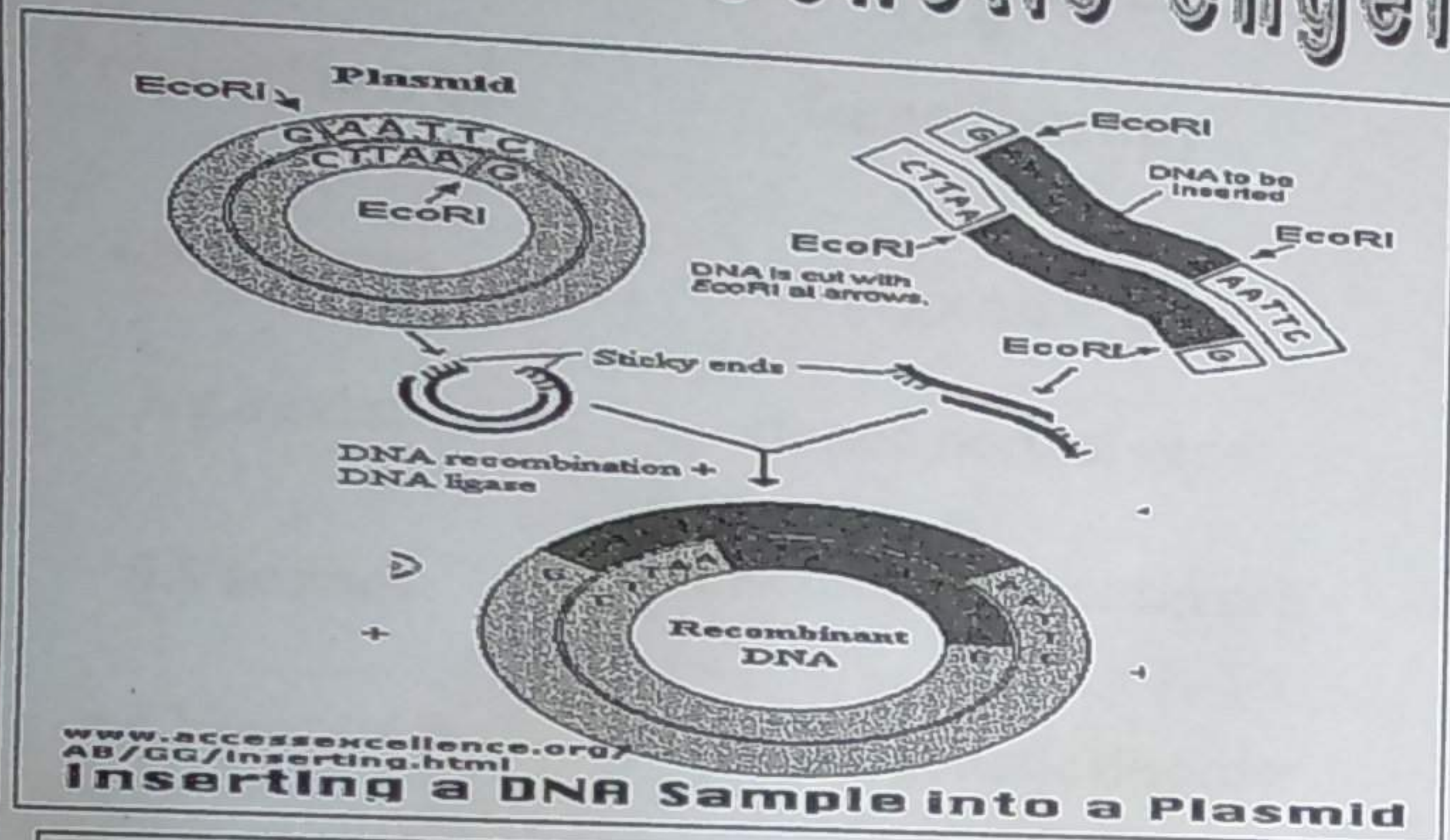
If inserted into a

*transmissible plasmid* (F Plasmid)

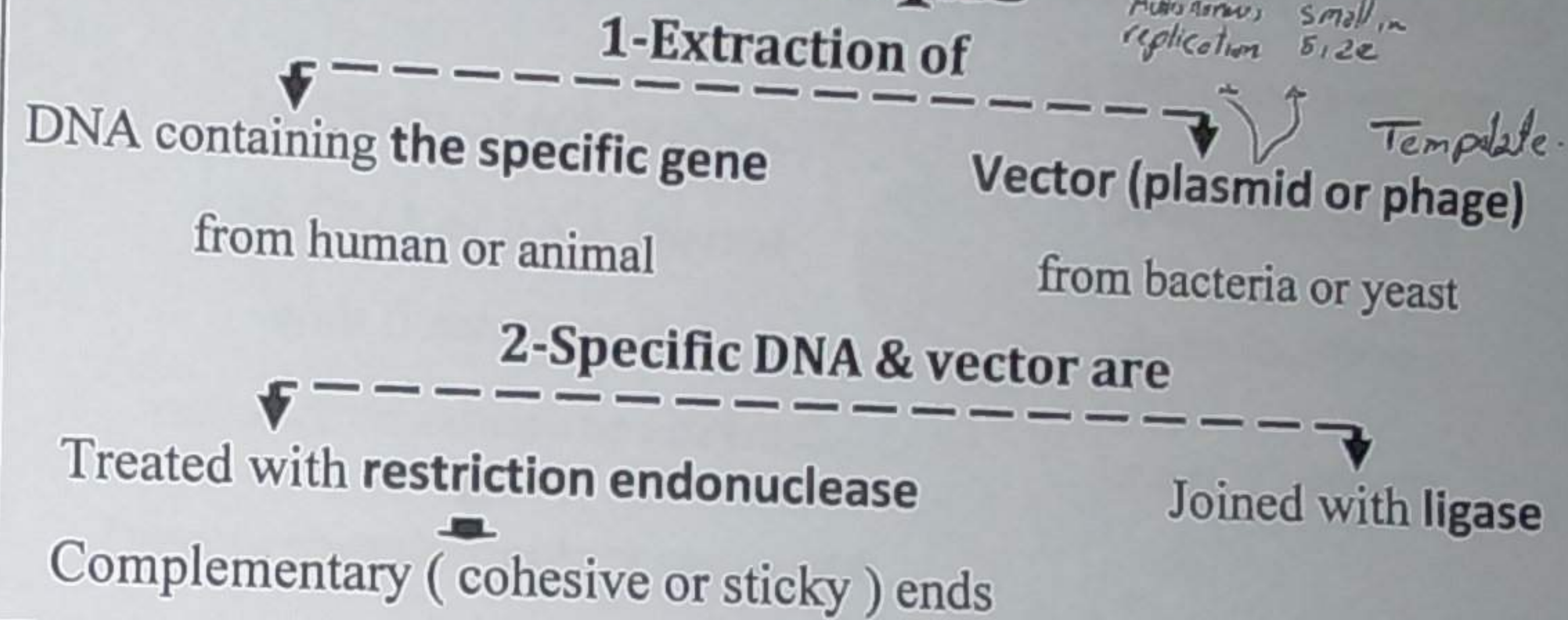
Disseminate through bact. population



# Genetic engineering (gene cloning)



## A-Technique



## Recombinant DNA molecule

Transferred into bacterial cell or yeast (host cell) by transformation

calc & heat shake

## 3-The cell multiplies

Creation of many clones

(genetically identical bacteria)

Each produces the gene product (a protein)

Harvesting the protein

from culture containing the clones

تطبيقات خريجه فوق آسين ت

## B-Applications

ضرر رطيم و انسولين و عالي بالجين و اعلى الجين

### Production of

#### i. Hormones

e.g insulin

#### ii. Vaccines

e.g Hepatitis B

### Gene therapy

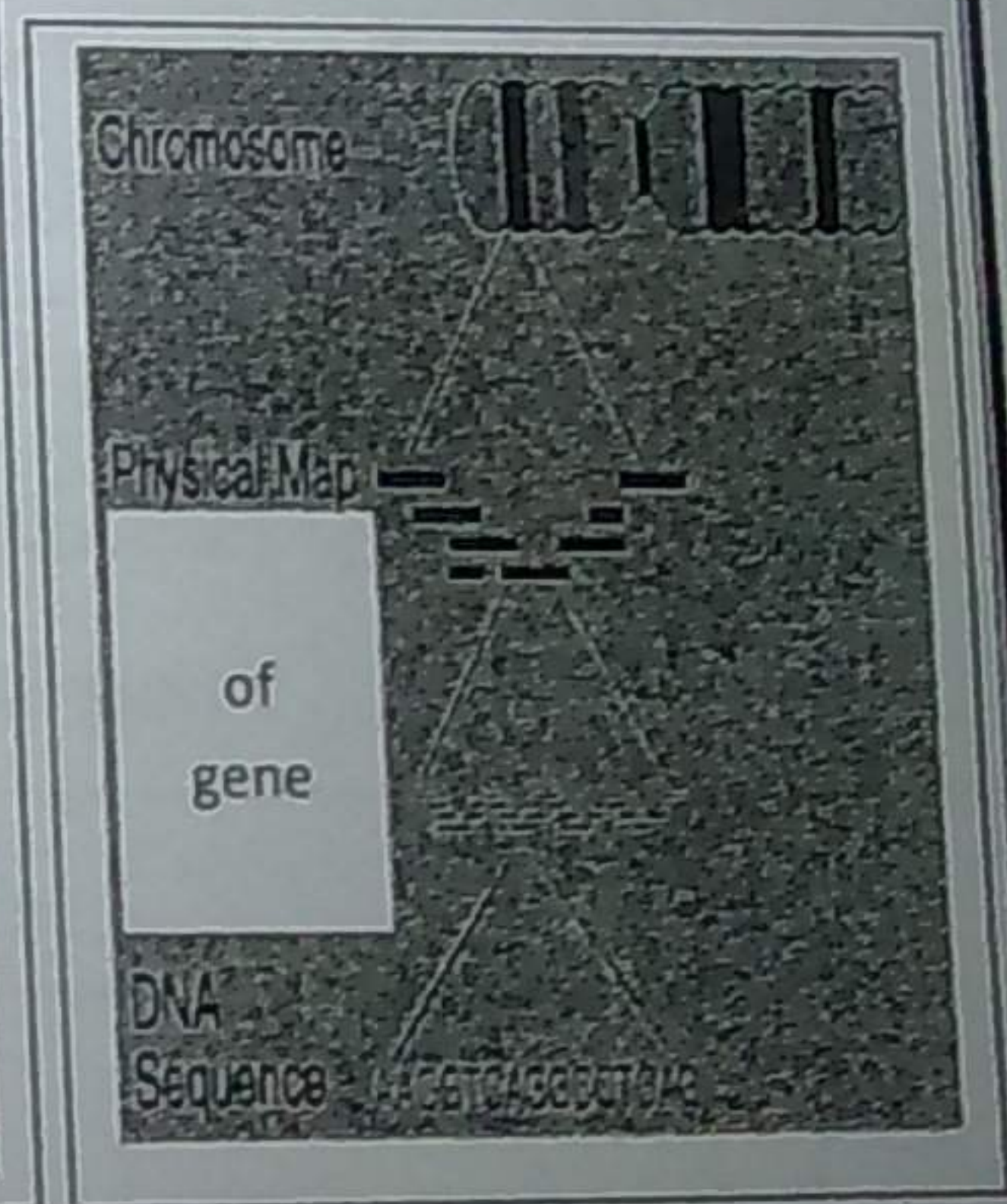
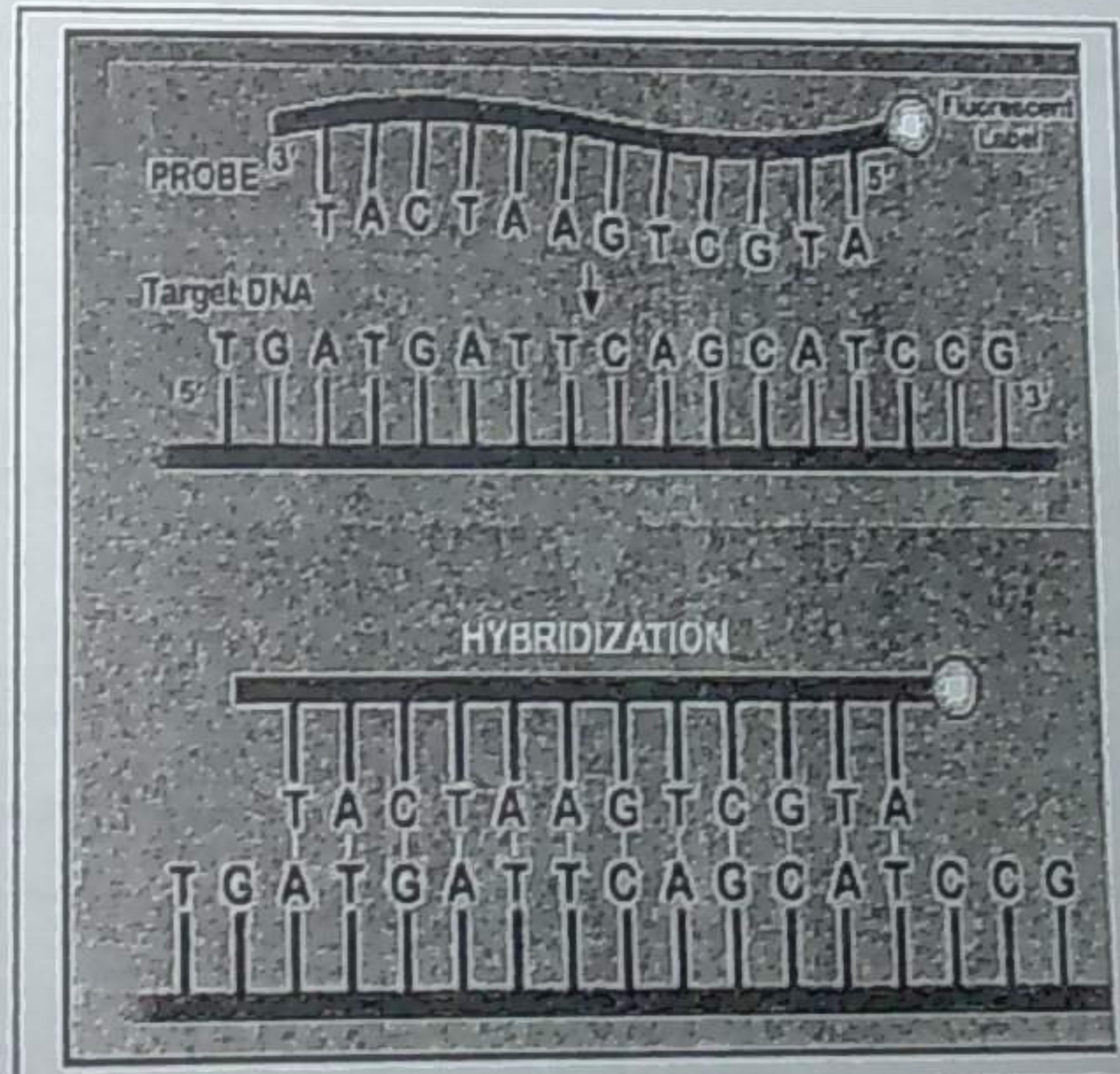
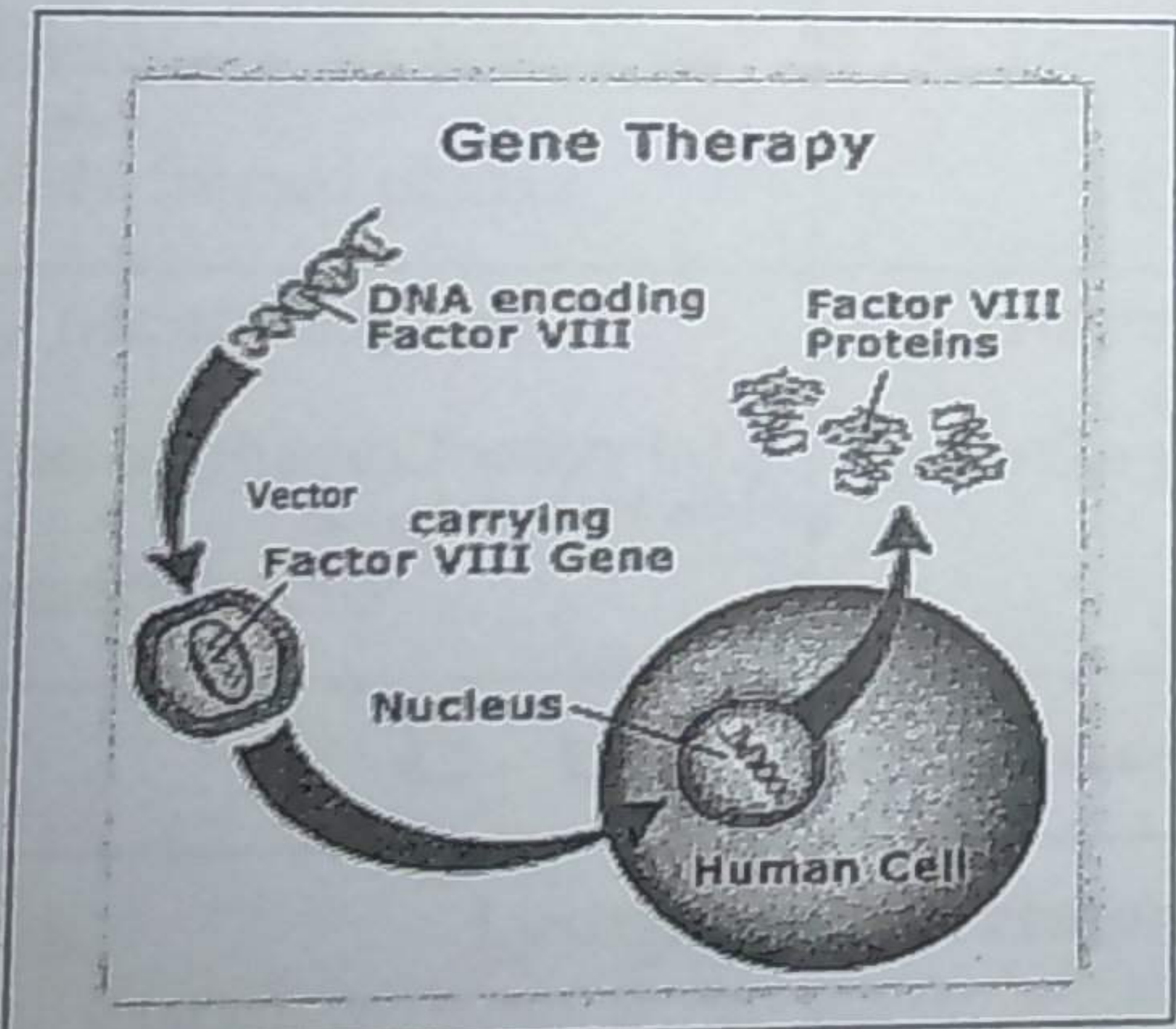
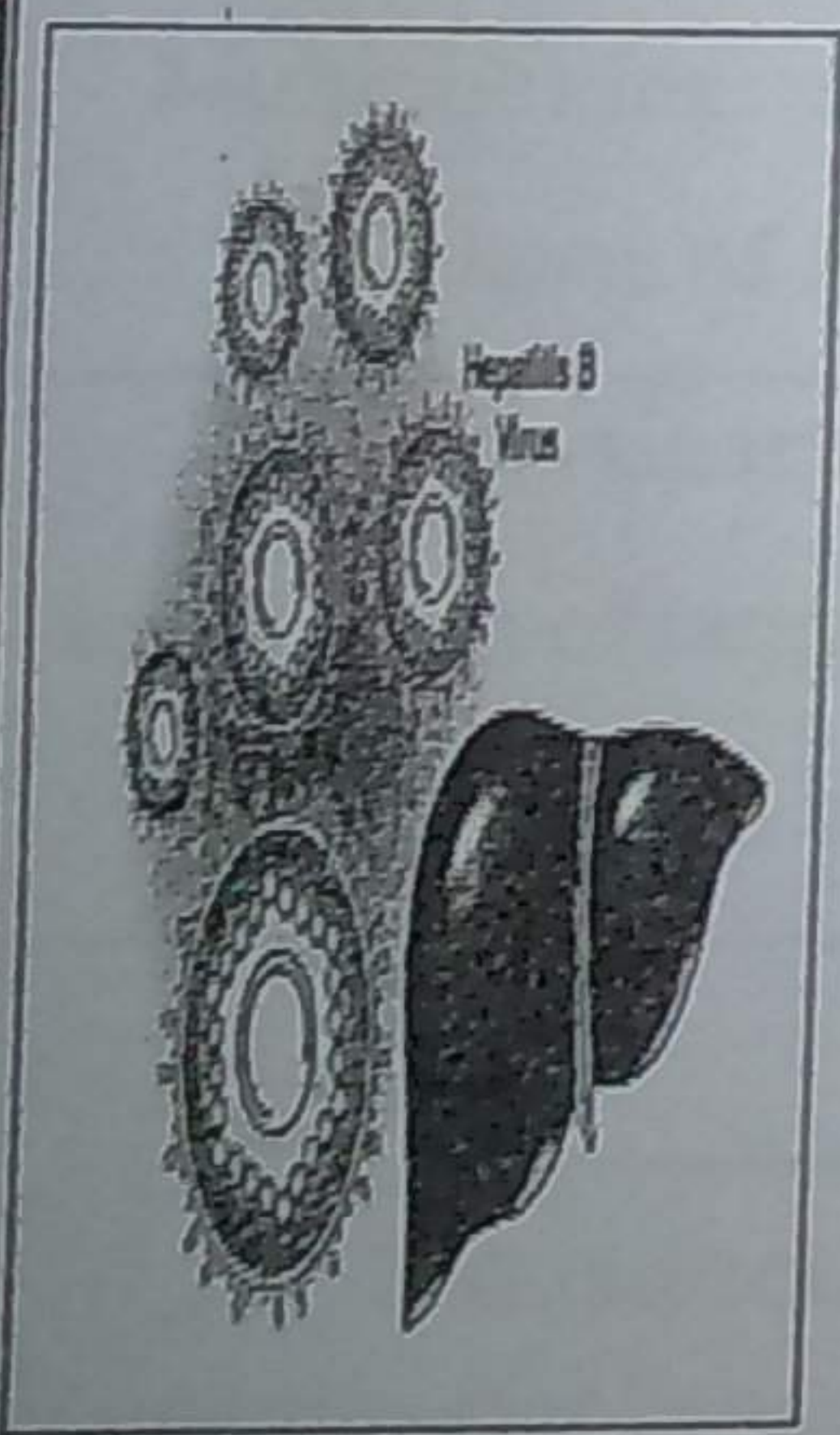
Preparation of  
cloned normal gene  
↓  
Insertion into human cell  
↓  
Correct genetic disorder

### Diagnosis of infectious diseases

Addition of **NA probe** (SS DNA or RNA labeled  
with fluorescent dye,  
radioactive isotope or enzyme)  
↓  
Detects **complementary sequence**  
of microbial gene  
↓  
**Hybridization**

### Chromosomal mapping & DNA sequencing

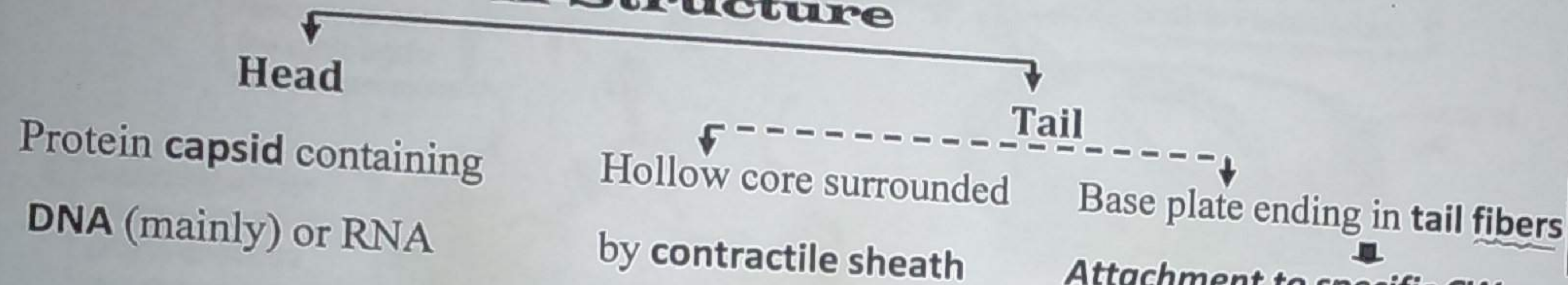
Determination of  
gene location  
in org.'s genome



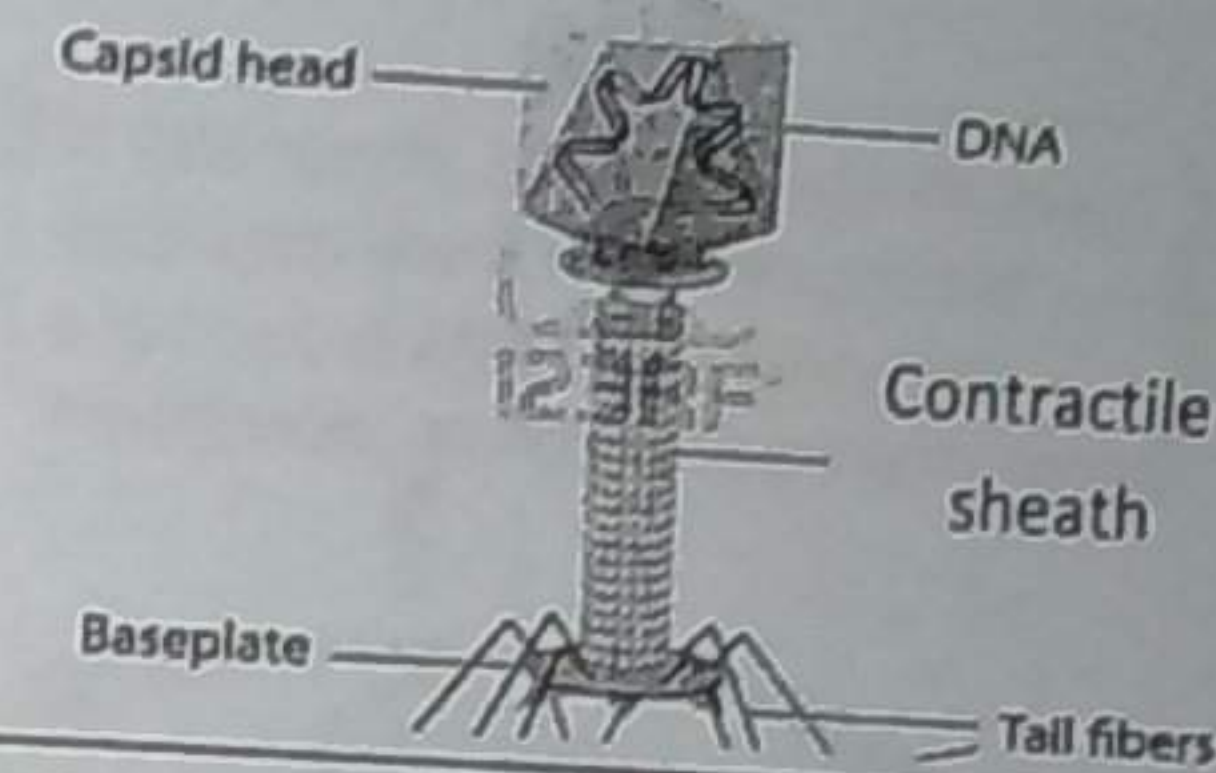
# Bacteriophage

VIRUS THAT INFECTS BACTERIA

## A-Structure



Structure of a Bacteriophage

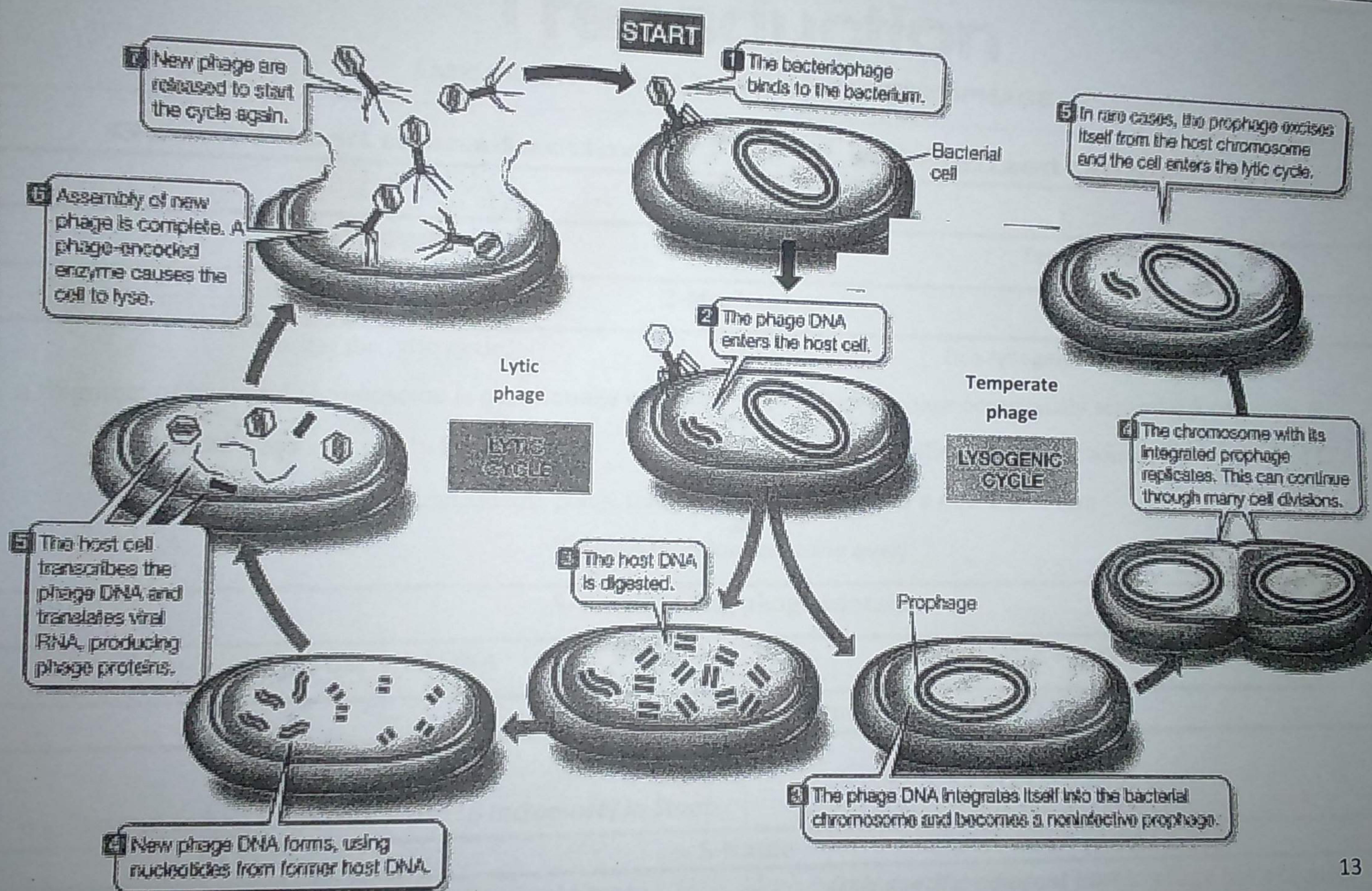


## B-Interaction between Bacteriophage & Bacterial host

Lysis	Lysogeny <small>مدمج بالخلل</small>
<p><b>Lytic (virulent) phage</b> infects bacteria</p> <p><small>virulence gene is active</small></p> <p>Remains <b>extrachromosomal</b></p> <p>Multiplies immediately → bacterial <b>lysis</b></p> <p>Release of newly formed phages</p>	<p><b>Temperate phage</b> infects bacteria</p> <p><small>virulence gene is inactive</small></p> <p>Integrates in bacterial chromosome, cd "<b>prophage</b>" (non lytic)</p> <p>No lysis ( no multiplication )</p> <p>Replicates with bact.chromosome &amp; transferred to progeny</p>
<p><b>NB.</b> Lysis may occur early due to <b>adsorption of large no of phages (heavy inf.)</b> onto bacterial cells</p> <p><small>rupture of cell membrane</small></p>	<p>Bacteria may acquire <b>new properties due to phage own genes</b></p> <p>e.g <b>Toxin production</b> by C.diphtheria &amp; Strept.pyogenes (erythrotoxic toxin)</p> <p>This is cd : "<b>Lysogenic conversion</b>"</p>

## C - Importance of bacteriophages

Transduction	Lysogenic conversion	Genetic engineering	Phage typing
Gene transfer between bacteria	( E )	Act as vectors	Identification of bacteria



# Transduction

Gene transfer between bacteria by BACTERIOPHAGE

## Generalized transduction

## Specialized transduction

### 1-Type of phage

Lytic ~~or temperate~~

Temperate

### 2-Mechanism

During the lytic cycle,  
a fragment of bacterial chromosome is **occasionally enclosed**  
**in the head** of a newly formed phage

In a **lysogenic bacterium**,  
the prophage occasionally separates **incorrectly** &  
carries a **specific fragment** of adjacent chromosomal DNA

The transducing phage transfers this fragment into a **new bacterium**

**Recombination (crossing over)**

### 3-Transducing phage contains:

Only **bacterial DNA**

Bacterial DNA + **part of phage DNA**

### 4-Transfer of plasmid

Yes

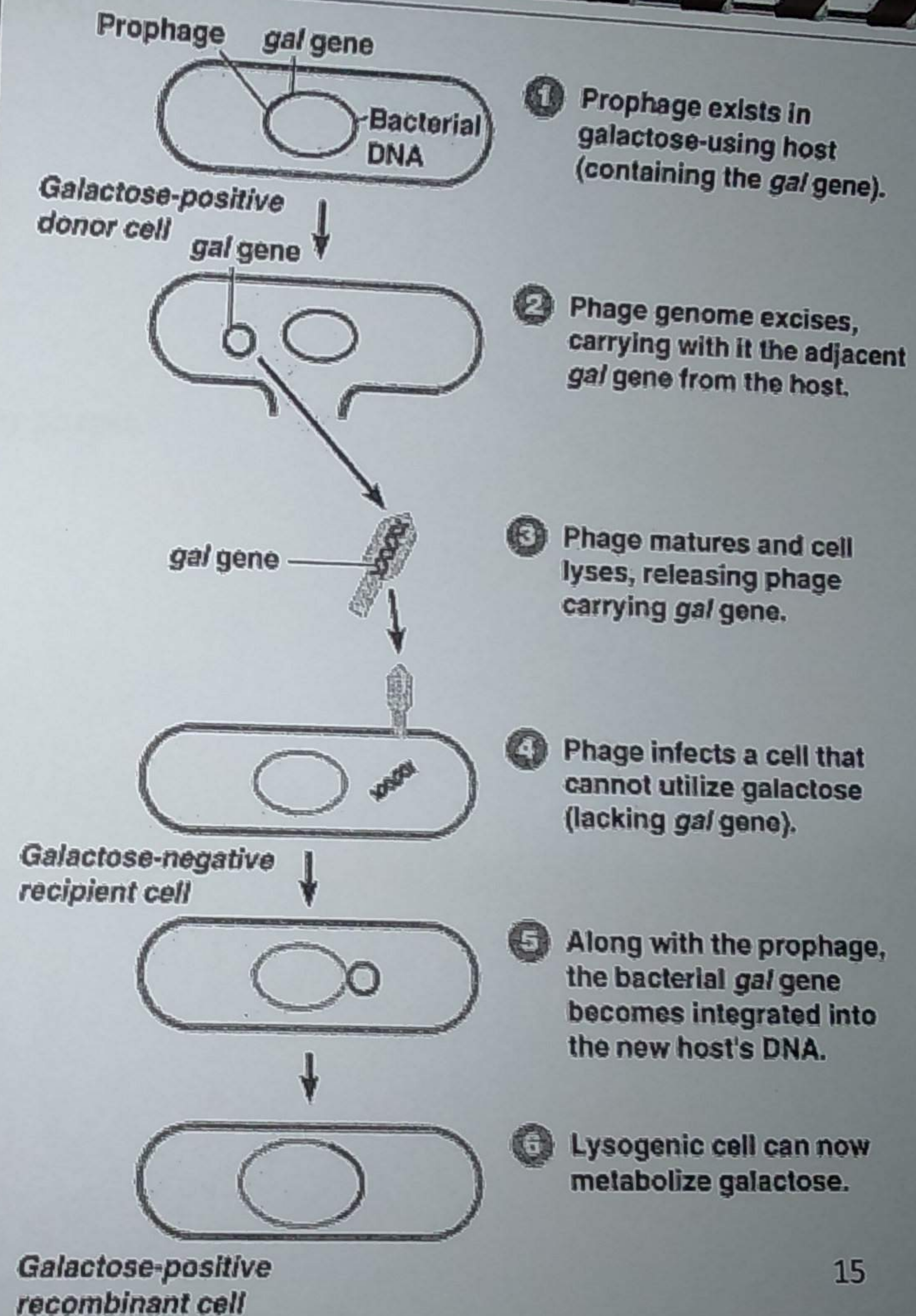
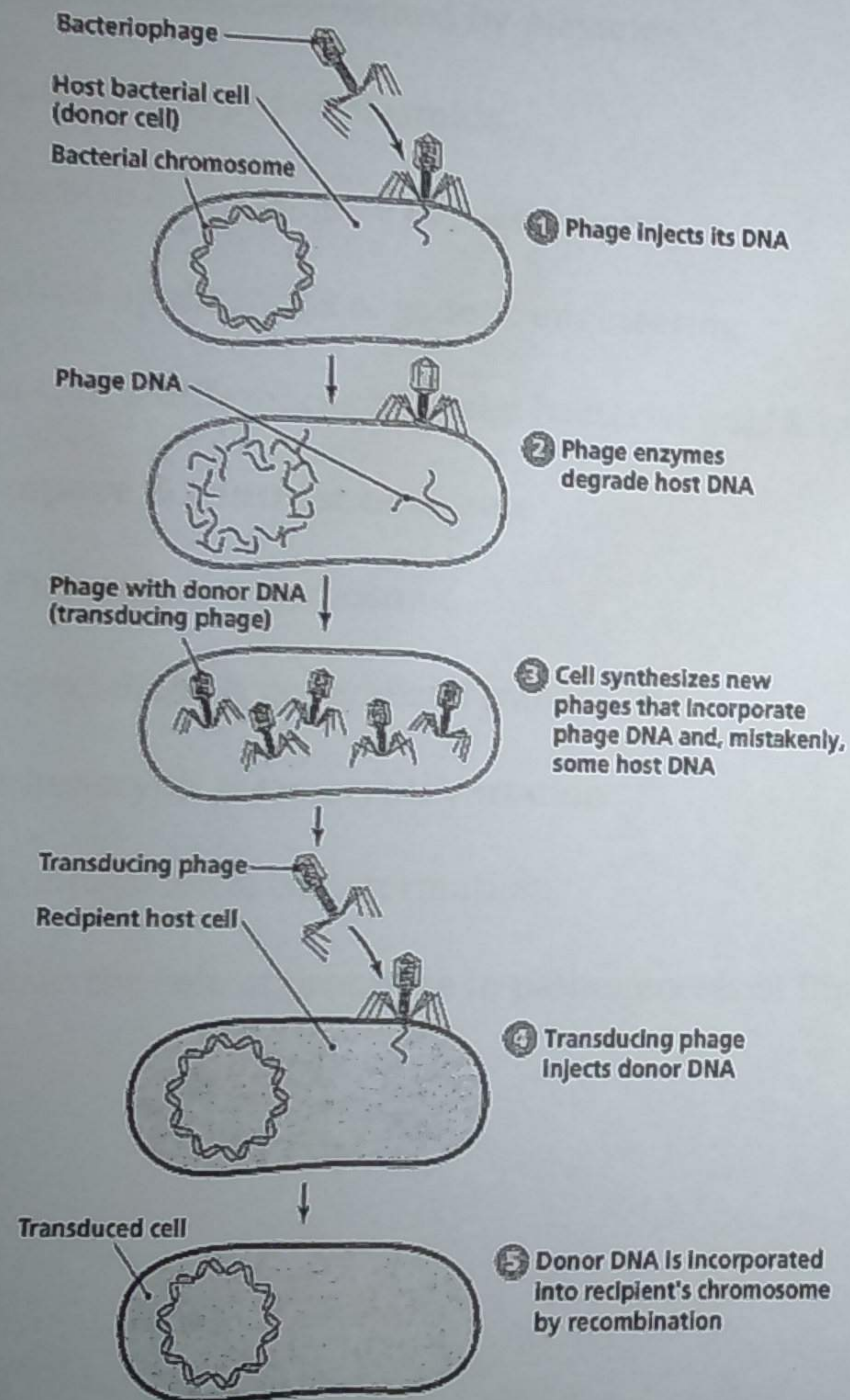
No

e.g Plasmid coding for *penicillinase* ( $\beta$  lactamase) in *Staph*

### 5-Name

**Any part** of bacterial DNA has an equal chance  
to be transduced

**Only specific adjacent part** to phage has a chance  
to be transduced



## Essay questions

- 1- Cell properties determined by plasmids.
- 2- Structure & types of plasmids.
- 3- Structure & importance of bacteriophage.
- 4- Medical applications of genetic engineering.
- 5- Mention 2 differences between bacterial lysis & lysogeny by phages.
- 6- **Compare & contrast between:**
  - a. Plasmids & transposons.
  - b. Specialized & generalized transduction.
  - c. Phenotypic & genotypic variation.
  - d. Conjugation & transformation.
- 7- Explain the role of prophage in pathogenesis of Diphtheria.

# General Bacteriology 5

ANTIMICROBIALS  
ANTIBIOTICS

# Antimicrobials

Substances that **kill**

OR

⊖ **growth & multiplication** of organisms

↙ In vivo

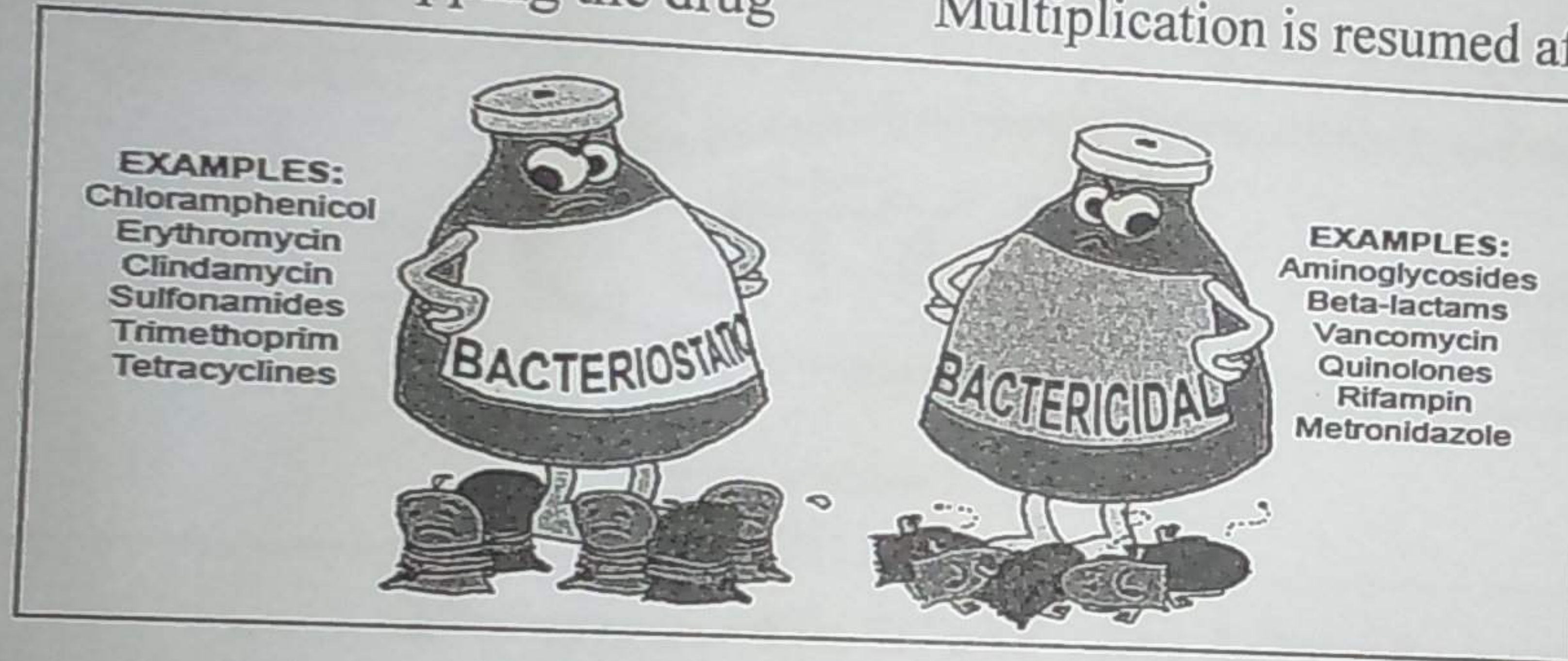
↘ Can be used systemically

**Cidal**

**Static**

No multiplication after stopping the drug

Multiplication is resumed after stopping the drug



## Classification of antibiotics

According to origin

According to spectrum

Naturally occurring

2ry metabolites of *living org.*

Bacteria

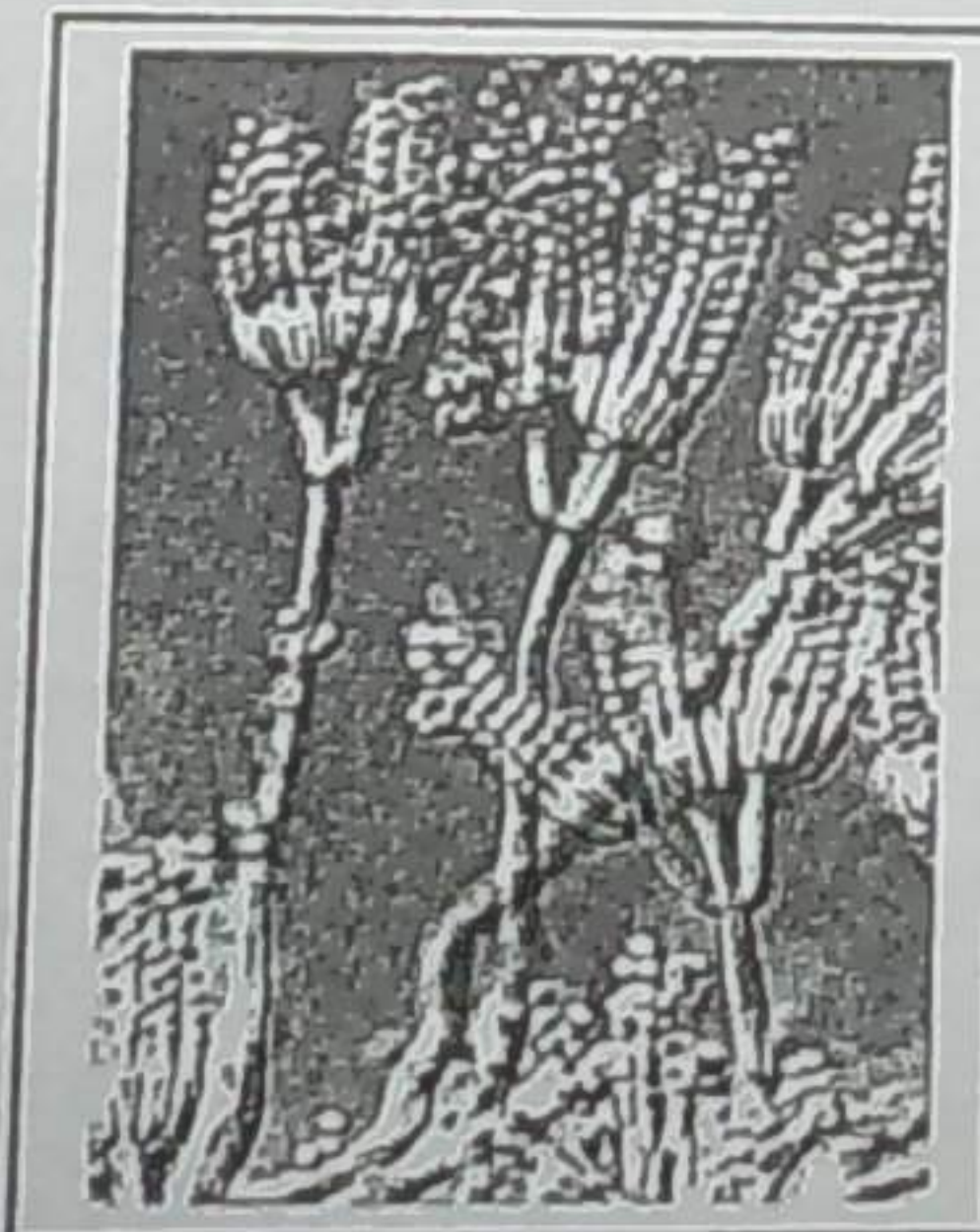
Molds

♥ Streptomyces

♣ Penicillium

♥ Bacillus

♣ Cephalosporium



Chemotherapeutics

Synthesized

in laboratory

Broad

Narrow

On *both*

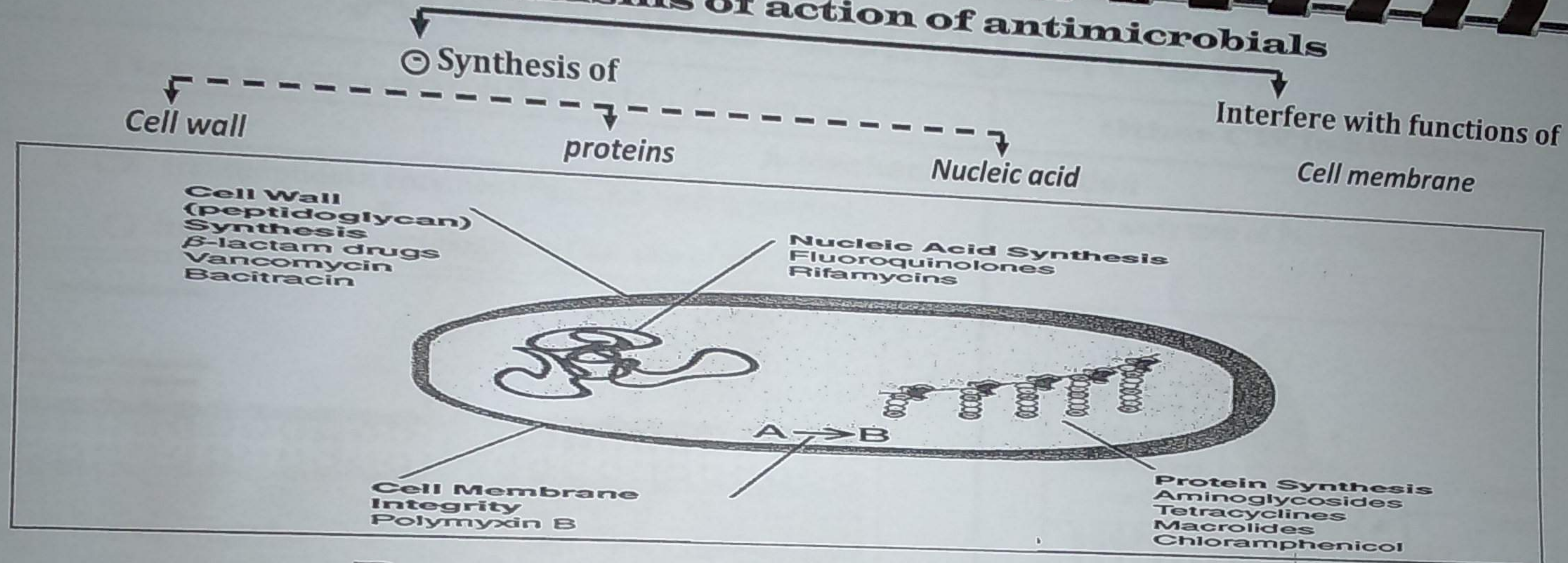
On *either*

Gram +ve  
&

of them  
only

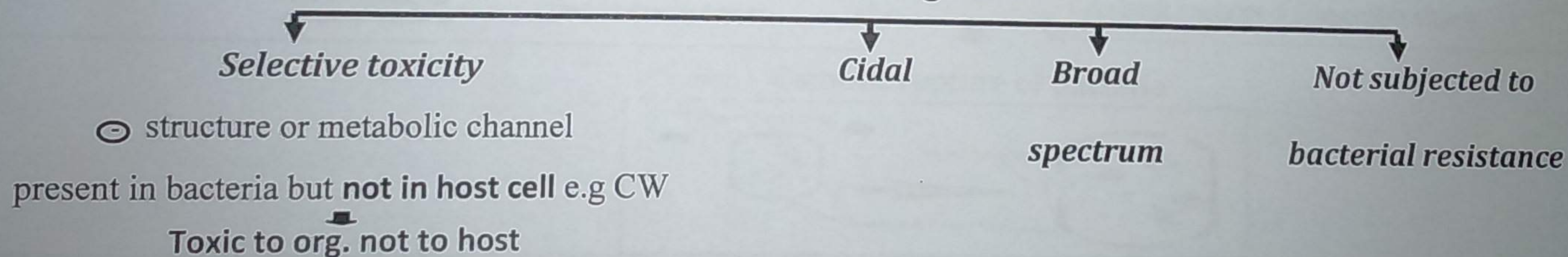
Gram -ve  
bacteria

## Mechanisms of action of antimicrobials

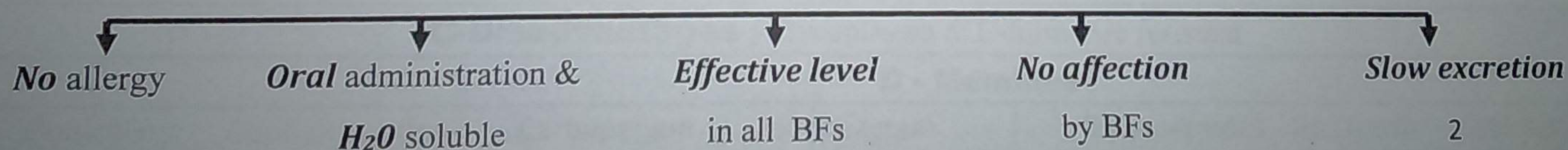


## Properties of an ideal antimicrobial

### A - Bacteriological



### B - Pharmacological



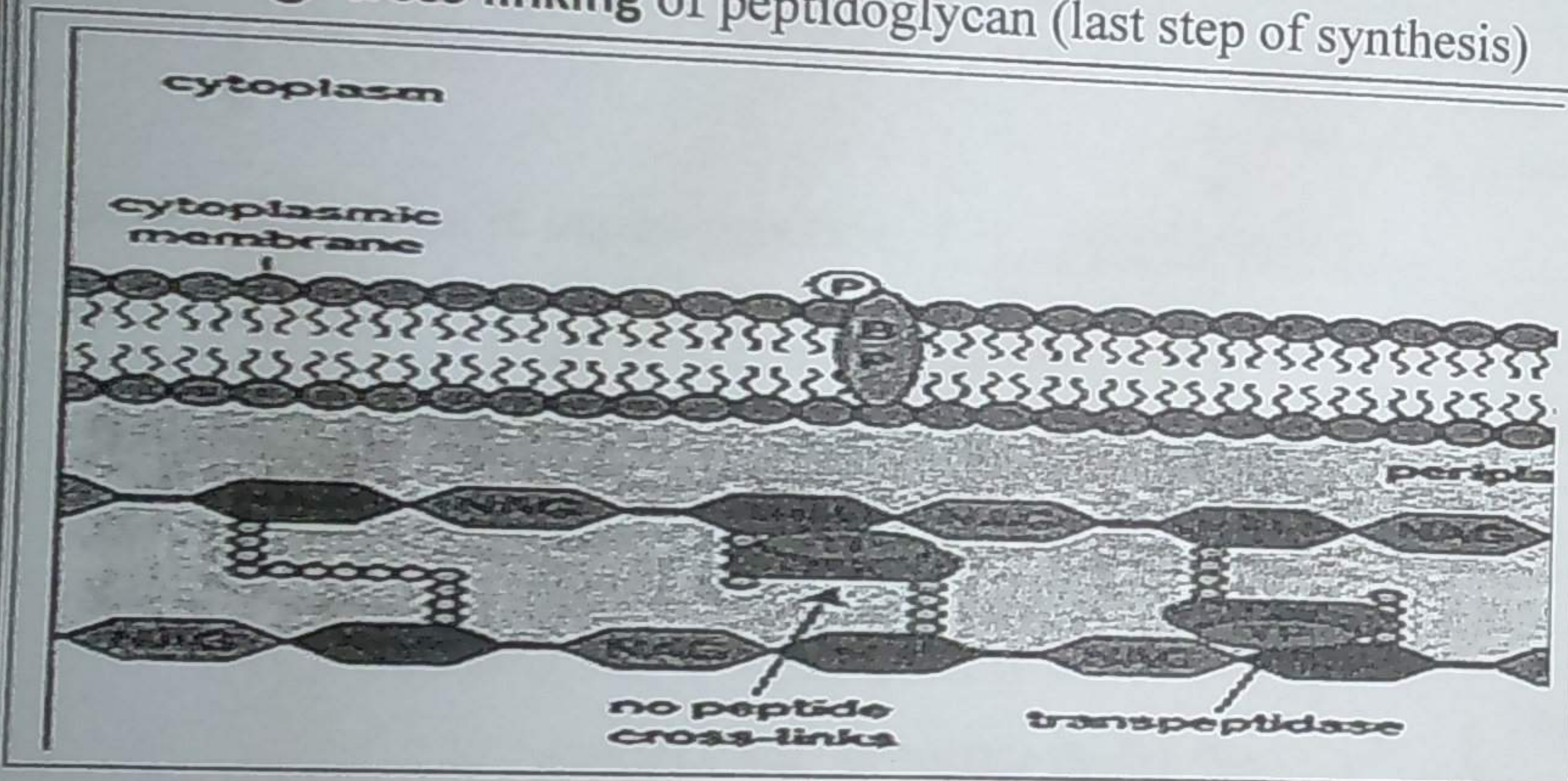
# Antibiotics acting on CW

$\beta$  lactam compounds (contain  $\beta$  lactam ring)

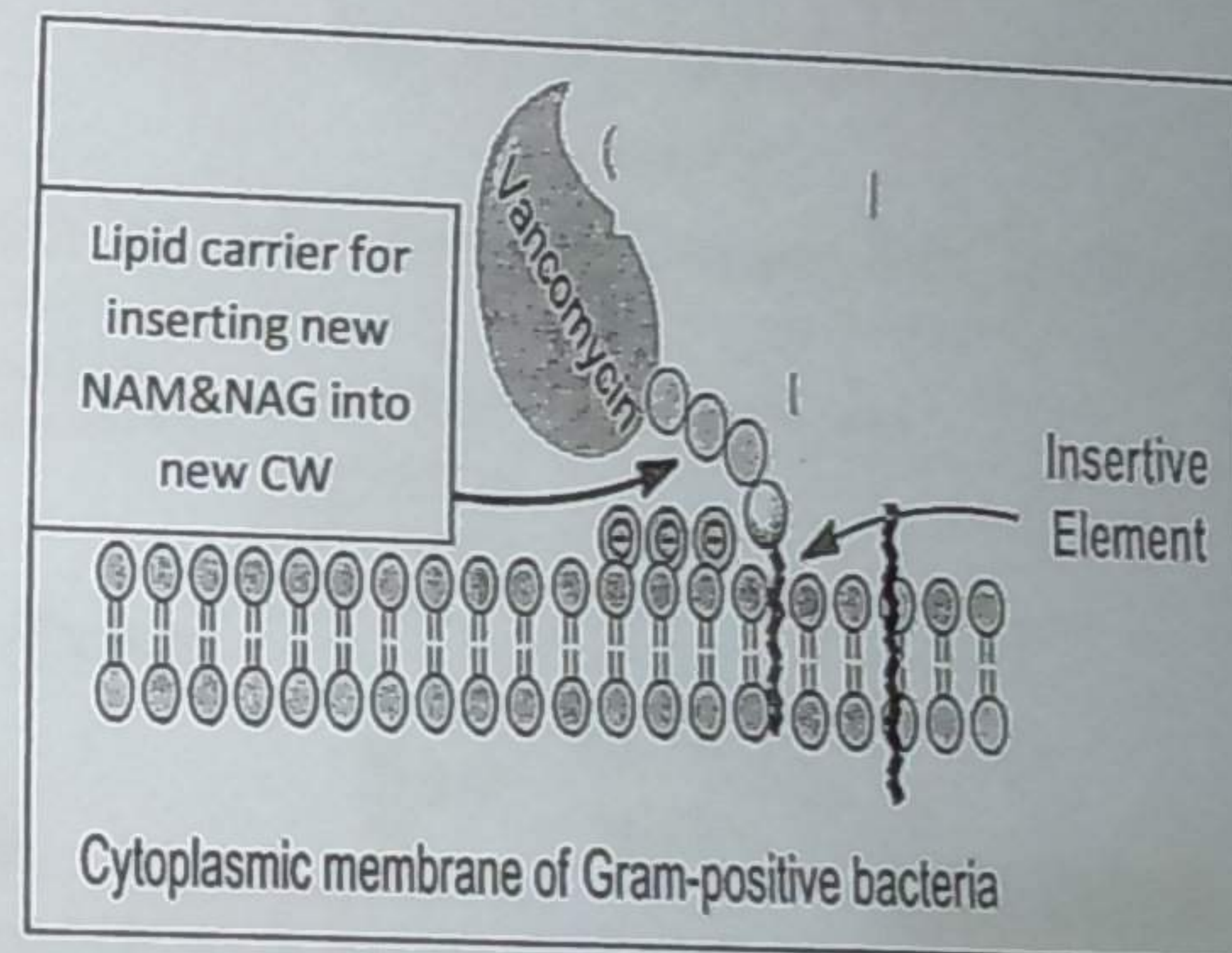
Other CW inhibitors

## A-Mechanism of action

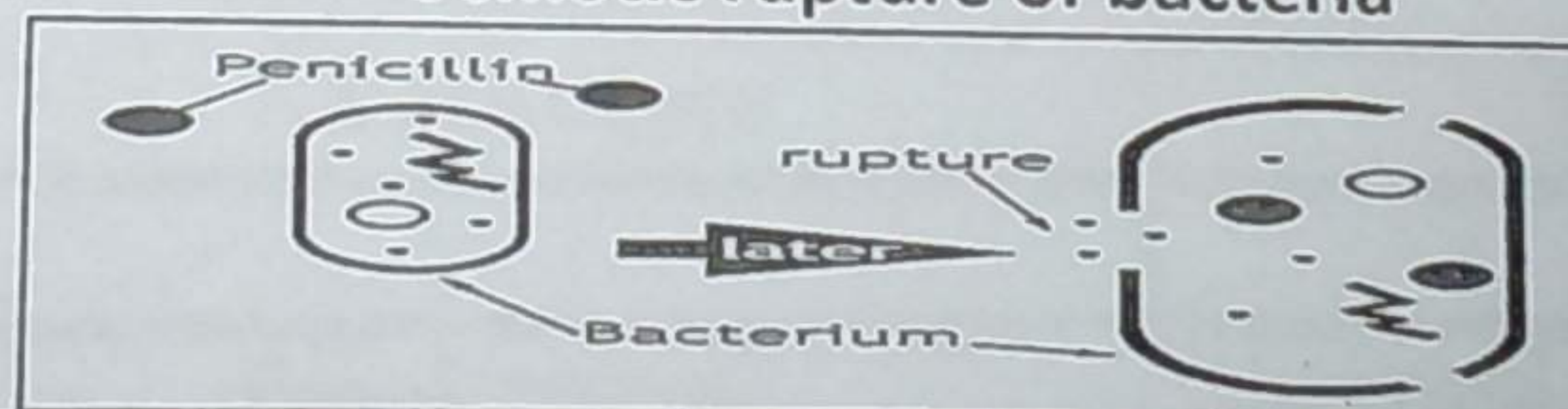
- transpeptidase enzymes ( Penicillin binding proteins)
- ↓
- cross-linking of peptidoglycan (last step of synthesis)



- early step of PG synthesis in CM



## Osmotic rupture of bacteria



**B- Advantages :** High selective toxicity; no harm to human cells which have no CW

**C-Disadvantages :** Mycoplasma & L-forms are resistant

## D - Members

Penicillins

Cephalosporins

Carbapenem

Monobactam

Glycopeptides

Bacitracin

Cycloserine

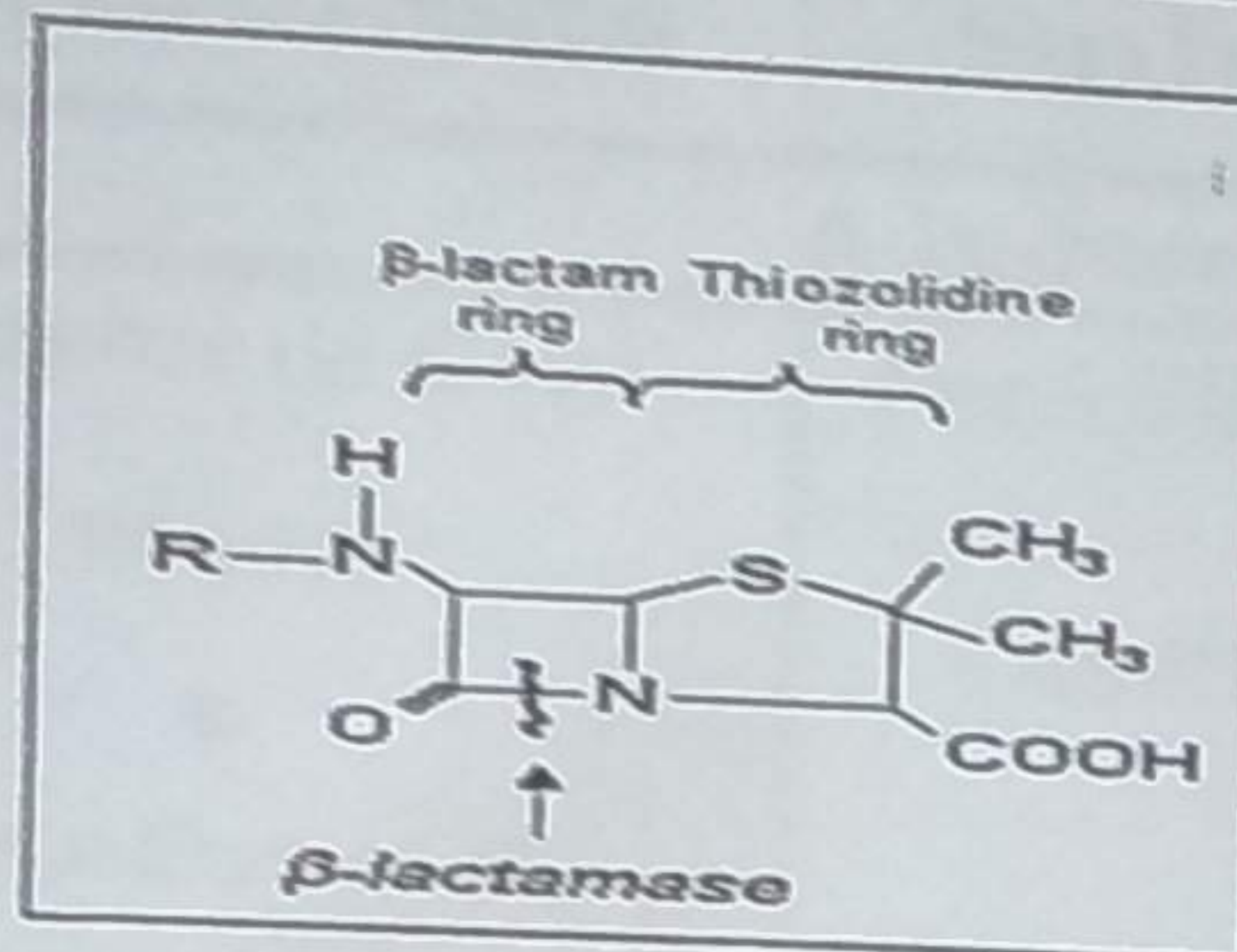
## E-Mechanisms of bacterial resistance

### 1-By destructive enzymes (coded by R plasmid)

$\beta$  lactamase (penicillinase)  
of Staph aureus

Destroy

Some penicillins & cephalosporins



ESBLs (extended spectrum  $\beta$  lactamase)  
of G-ve bacilli

(arise by mutation in genes  
on plasmids coding for  $\beta$  lactamase)

destroy

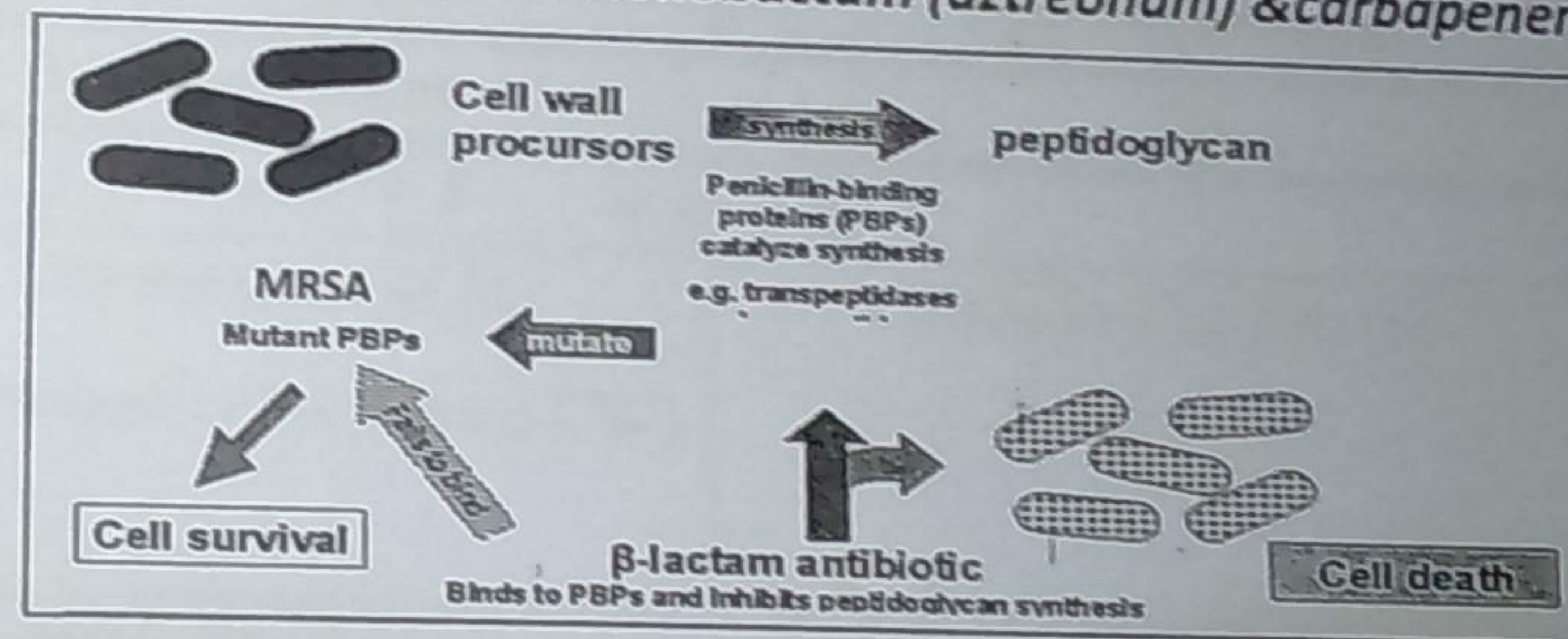
As before + monobactam (aztreonam) & carbapenem

### 2- Alteration of transpeptidase

By MRSA (Methicillin resistant Staph. aureus)

Resistant to all  $\beta$  lactams,

including those resistant to  $\beta$  lactamases e.g  
methicillin, cloxacillin & nafcillin



### F- Important notes

	Advantages	Disadvantages
<b>1-Penicillins</b>		
i. Penicillin G, ampicillin, amoxicillin	Broad spectrum	Destroyed by $\beta$ lactamase (penicillinase)
ii. Methicillin, cloxacillin, nafcillin	Not destroyed by $\beta$ lactamase	Narrow spectrum: G+ve only
<b>2-Glycopeptides</b> Vancomycin & teicoplanin	Only TTT of MRSA	Narrow spectrum: G+ve only

# Cell wall deficient bacteria

Cell wall deficient bacteria			
Mycoplasma	Protoplasts	Spheroplasts	L-forms
A-Inducer			
Natural (no inducer)	Lysozyme on G+ve bacteria ↓ Destruction of PG	Penicillin on G-ve bacteria ↓ ⊖ synthesis of new PG	i. Penicillin on G+ve or -ve bacteria ↓ ⊖ synthesis of new PG ii. Spontaneous
B-Structure & Characters			
L a c k   r i g i d   c e l l   w a l l			
i. No defined shape ii. Resistance to antibiotics acting on CW	Complete absence of CW	Remnants of PG (damaged or weakened CW)	Vary in size & shape
C-Osmotic sensitivity			
NO (stable)	Sensitive → vary in size with OP of suspending medium		More stable
D-Multiplication			
Yes	No	Yes	Yes
E-Reversion			
No		5	Yes, if penicillin is removed ↓ Relapse of infection

# Mycoplasma

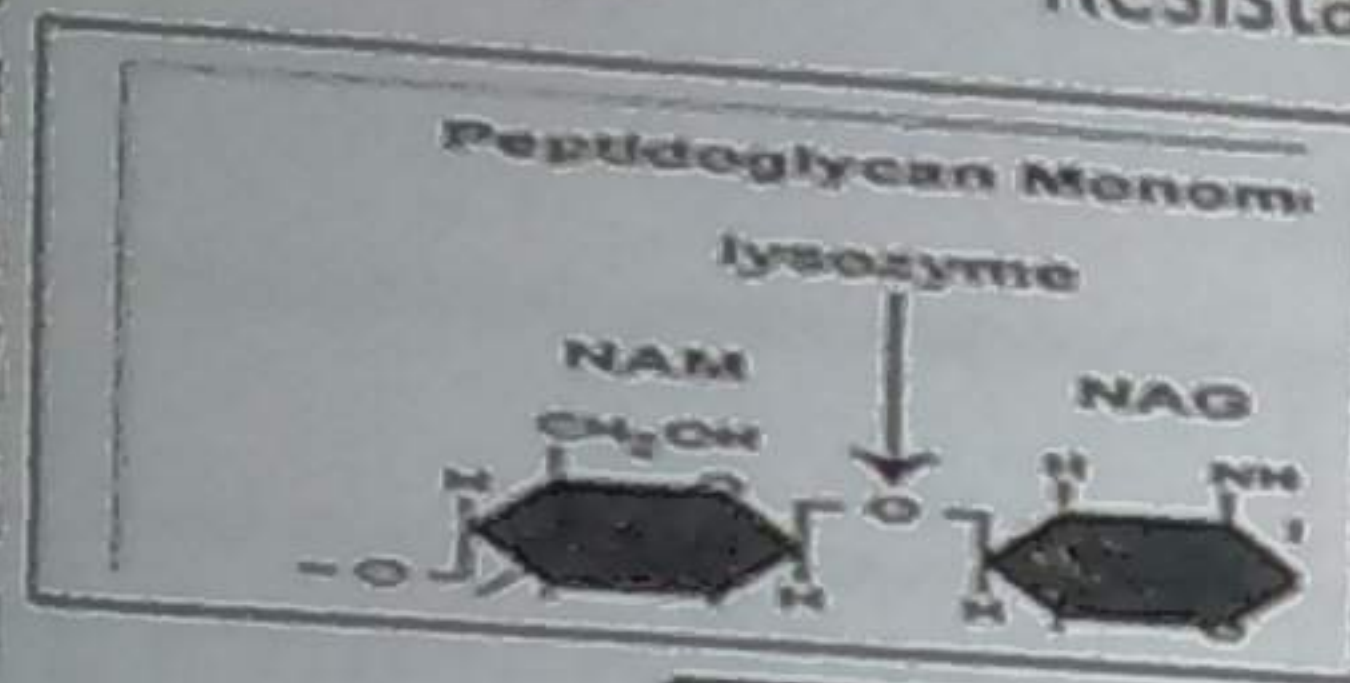
Natural absence of CW

No shape

Resistance to antibiotics on CW

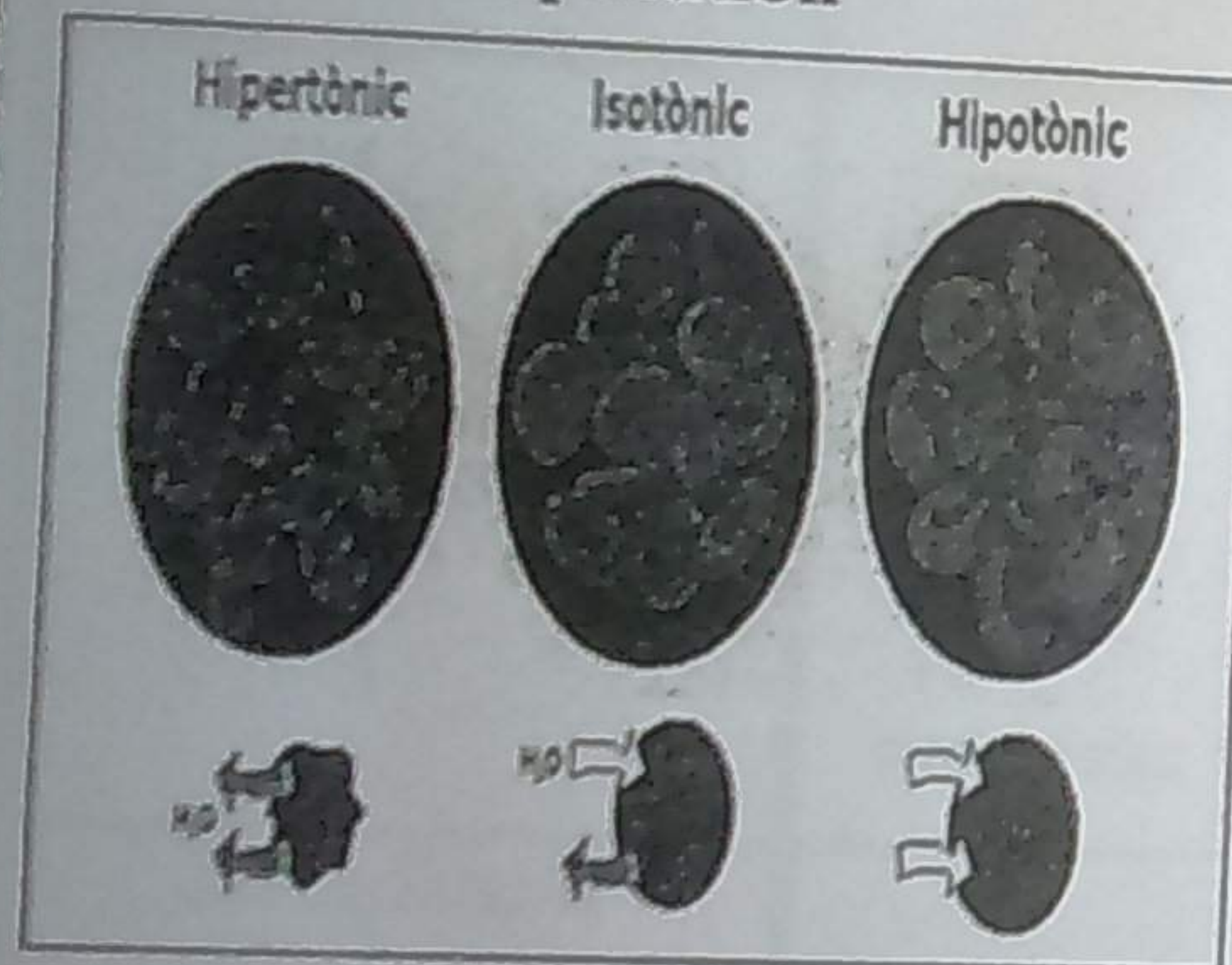
No reversion

Compensatory ↑ in CM thickness  
Osmotic stability → Multiplication



Lysosymes on G +ve in vitro → Destruction of PG & teichoic acid → No CW

No multiplication



Osmotically sensitive

Isotonic M

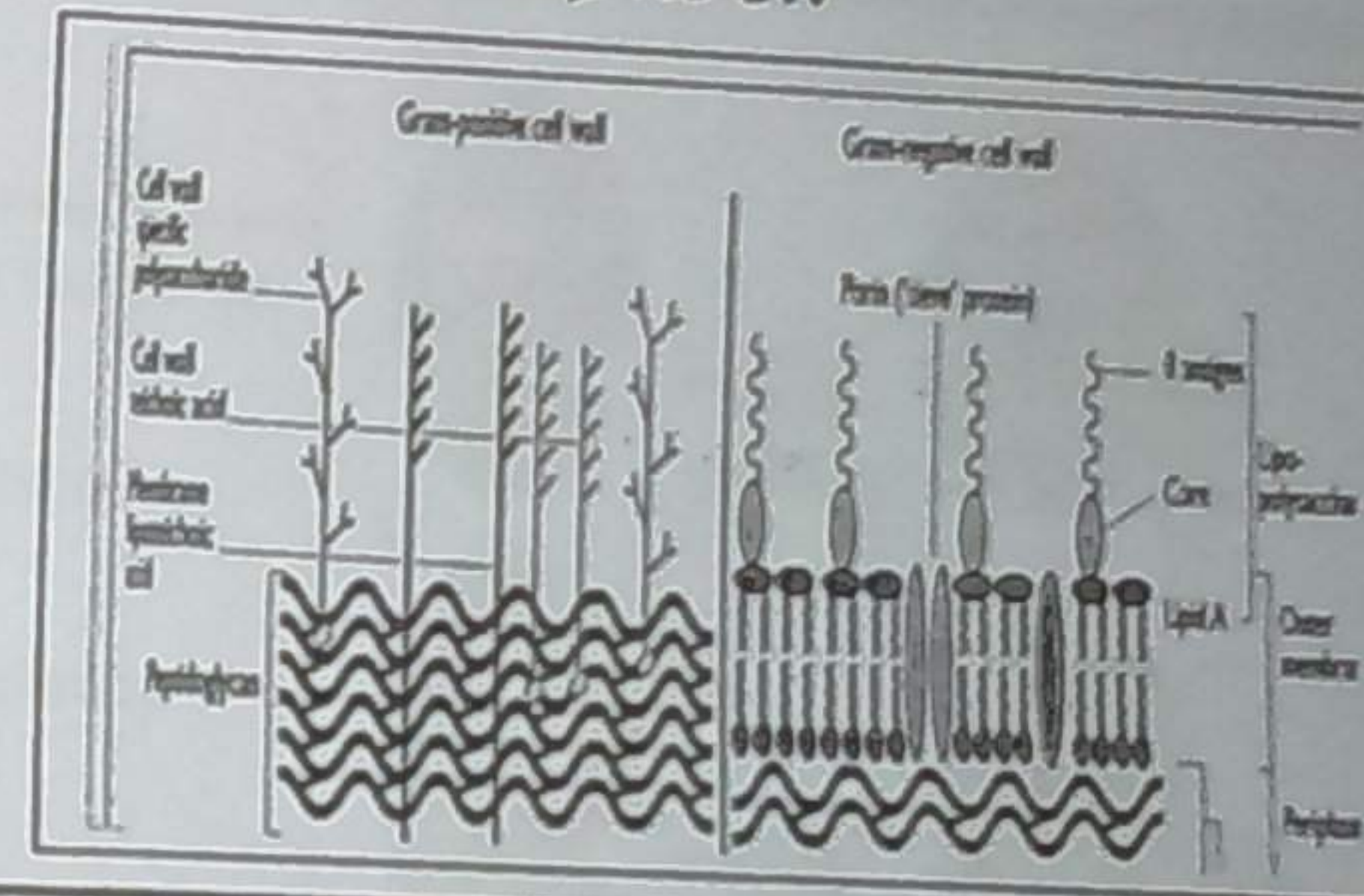
Hypotonic

Hypertonic M

No rupture

Ballooning & rupture

Shrinkage



Spheroplasts

Penicillin on G-ve in vitro → ⊖ CL of PG

Loose (remnant) PG

Osmotically sensitive a.s. (protoplast)

Remaining CW layers are intact (OM)

Multiplication (if maintained in isotonic H)

L (Lister institute) forms

Rarely Penicillin on G +ve or G -ve → partial ⊖ of CL of PG in few bacteria during infection

Part of PG remains intact & remaining CW layers are intact

Osmotically more stable

Survive (although the body is Hypotonic) & multiply (new L-forms) for sometime

Continuous use of Penicillin

Kill them

Early stoppage of penicillin



CW revert to original form → relapse

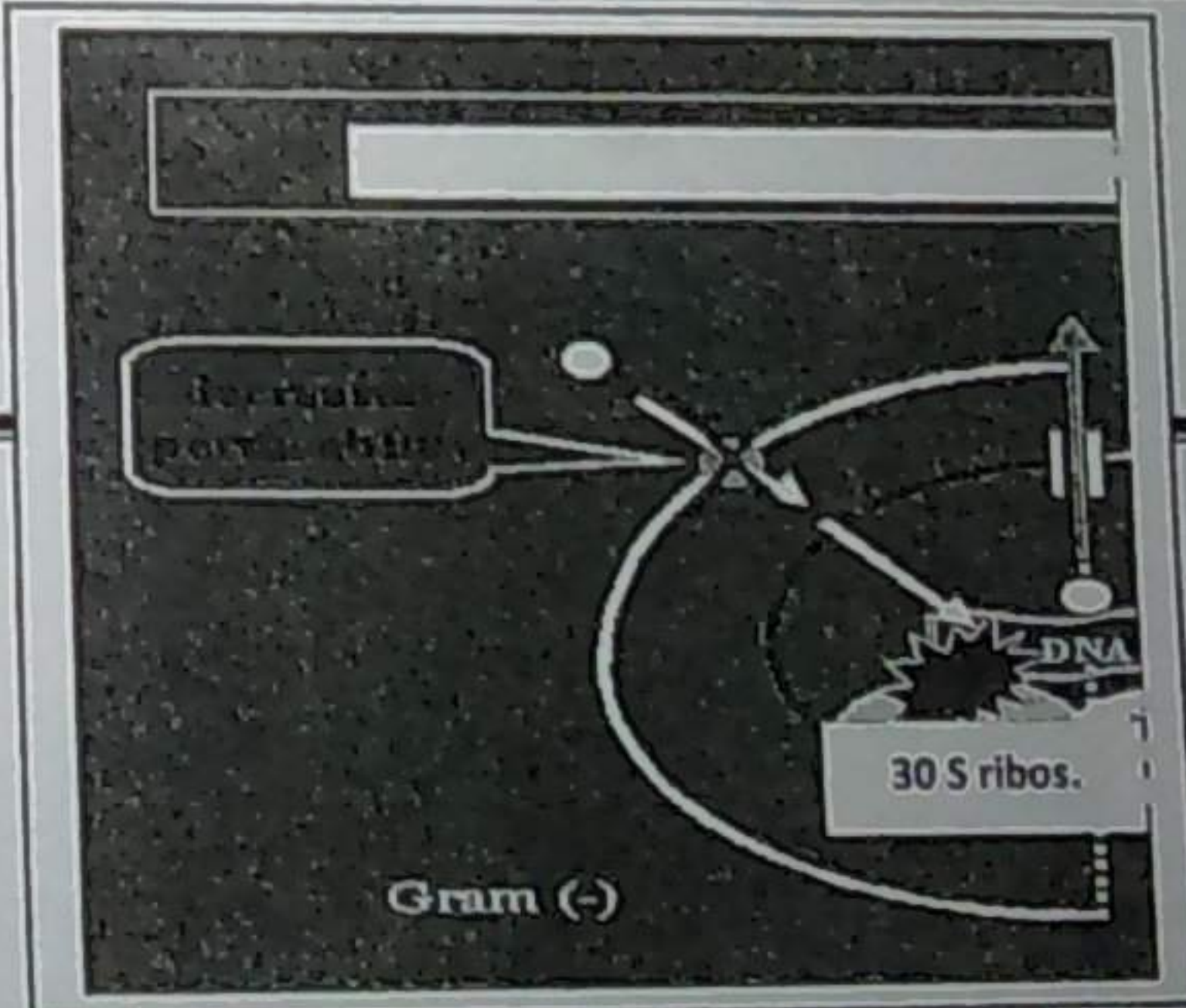
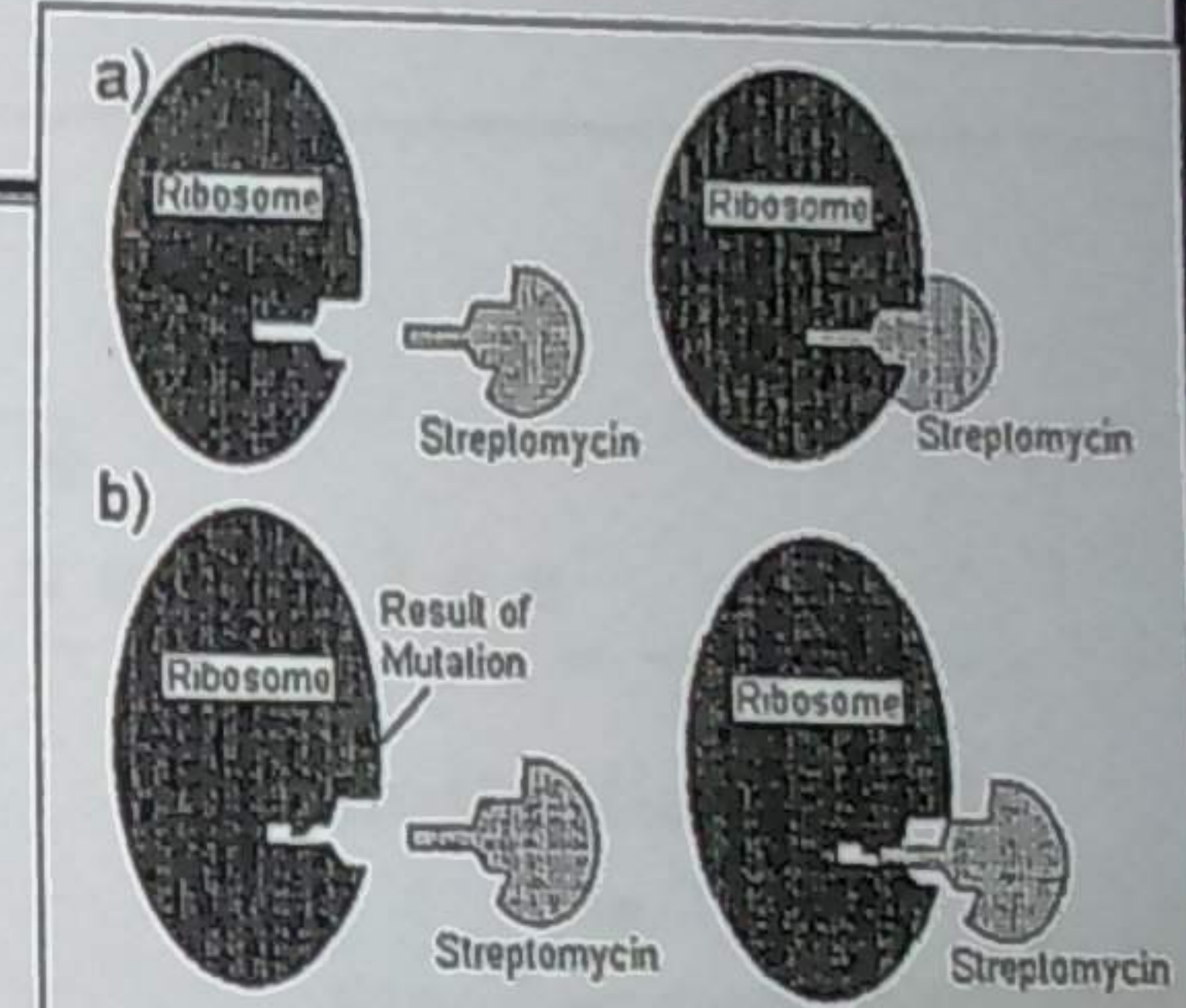
# Antibiotics inhibiting protein synthesis

## Advantages


Selective toxicity : Bacterial ribosomes are different from human ribosomes

### A- Drugs acting on 30S ribosomes

	S.E.		Mechanisms of bacterial resistance
1-Aminoglycosides	Nephrotoxic		
i. Streptomycin	Toxic to 8 <sup>th</sup> CN ↓ deafness		Change of streptomycin receptor (target R) on 30S ribosome  (due to spontaneous mutation of chromosomal gene)
ii. Amikacin			↓ permeability to the drug  Change in OMP ↓ ⊖ of active transport of drug
2-Tetracyclines	Permanent staining of child teeth  (if given in pregnancy or early in life)		As amikacin



## B - Drugs acting on 50S ribosomes

	S.E.		Mechanisms of resistance
1-Chloramphenicol	BM depression		<i>Destruction by acetyl transferase</i>
2-Macrolides (erythromycin)			
3-Azalides			
4-Clindamycin	Superinfection → pseudomembranous colitis		

### Superinfection

#### Etiology

Prolonged use of BS antibiotic to treat pathogenic bacteria

↓  
Suppression of sensitive bacterial flora

↓  
Overgrowth of flora which are

↙  
*Resistant*

↘  
*Potentially pathogenic*

#### Examples

↓  
*Candida*

↓  
*Oral thrush  
or vaginitis*



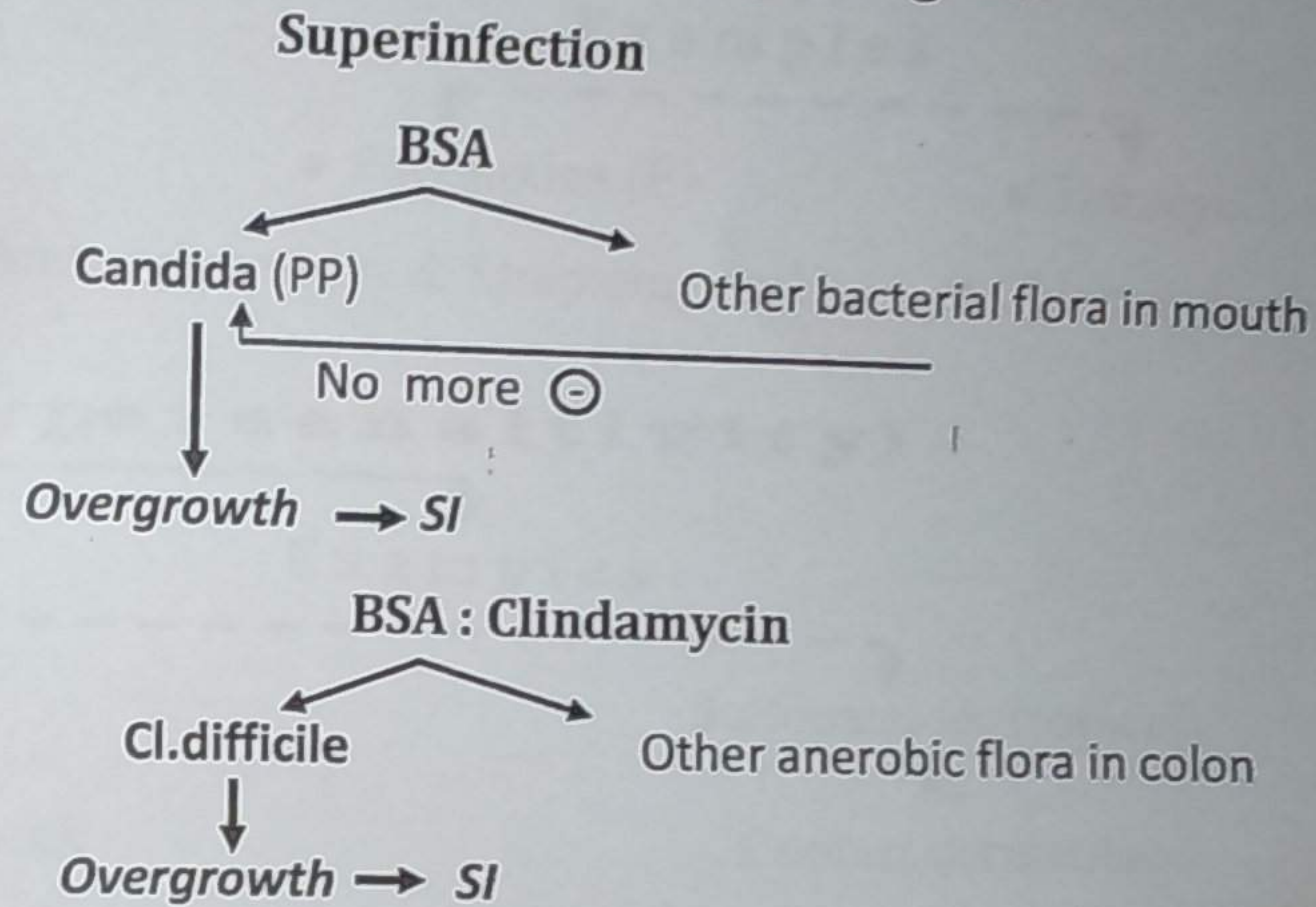
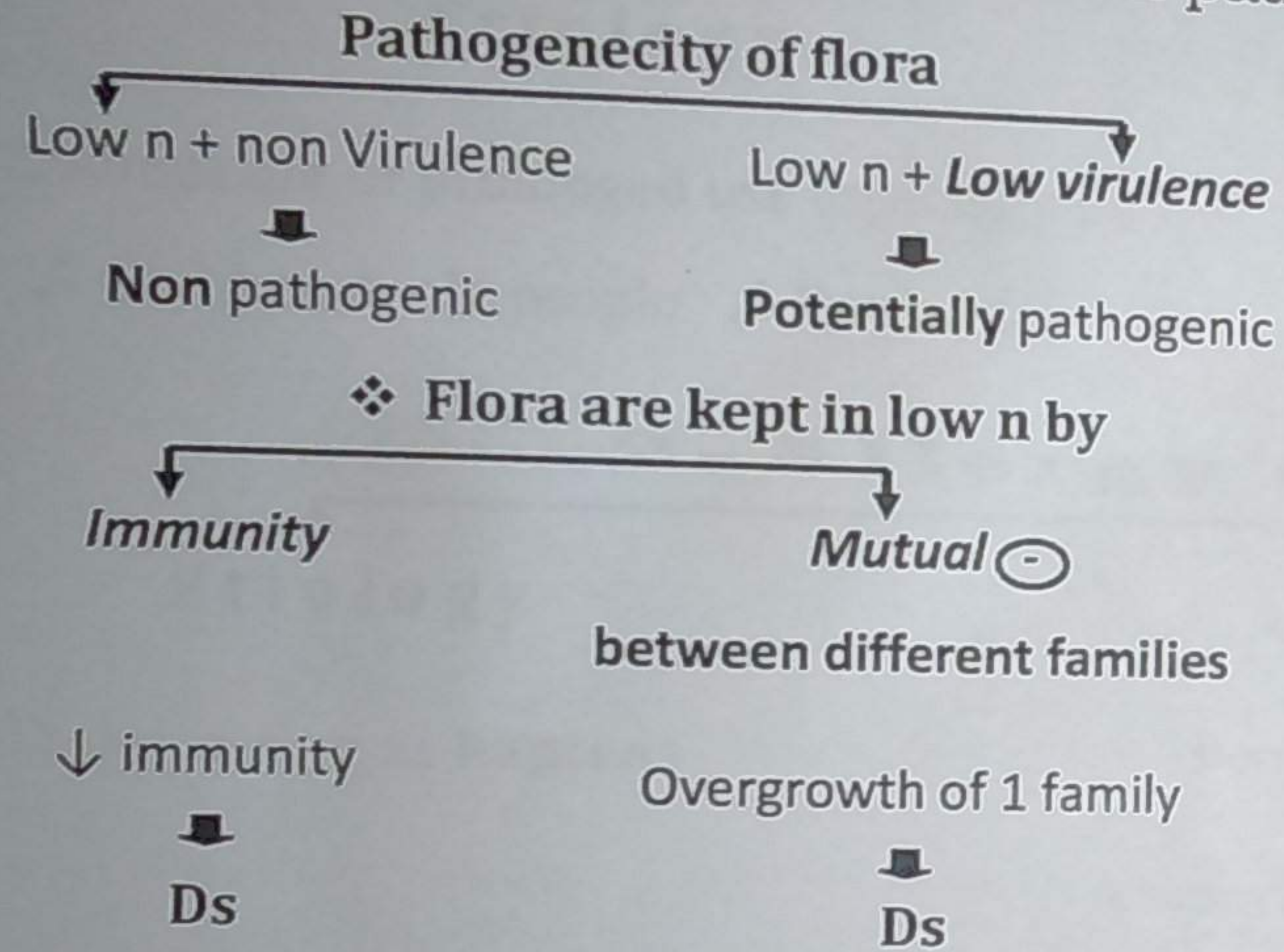
↓  
*Clostridia difficile*

↓  
*Pseudomemb.colitis  
(drug associated colitis)  
↓  
Diarrhea*

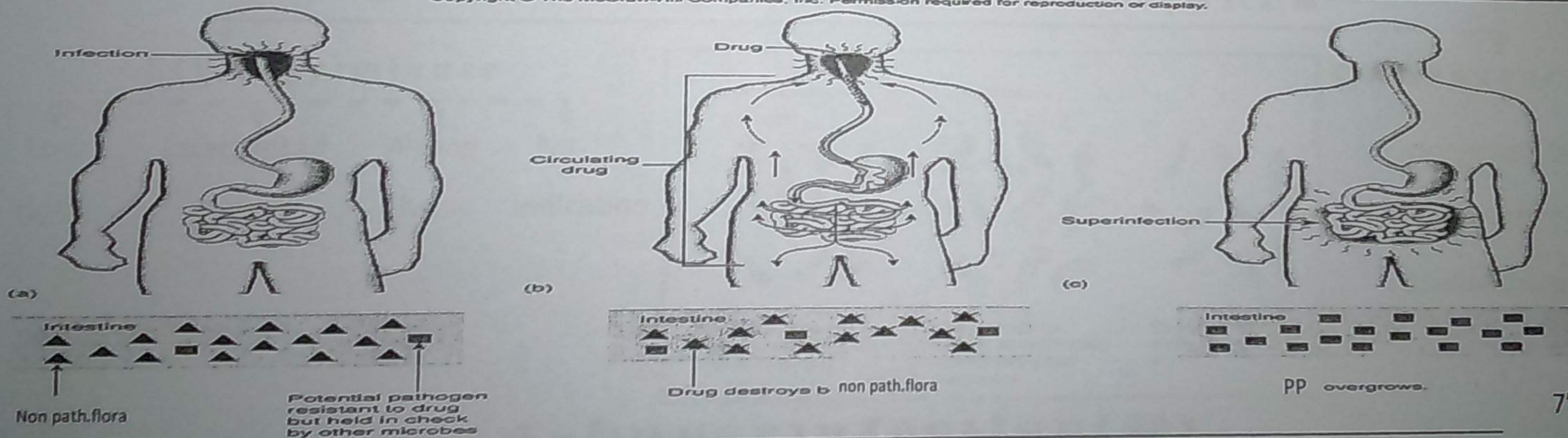


# Flora

- ✓ Organisms that grow in healthy persons in many sites e.g Skin ,GIT,vagina
- ✓ Some are non pathogenic & others are potentially pathogenic



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# Complications of chemotherapy

## 1-Drug toxicity

### Etiology

Overdosage or prolonged use especially in:

♪ Children & old people    ♫ Pregnant ♀

### Examples

- Polymixins (E)
- Aminoglycosides & Streptomycin(E)
- Tetracyclines (E)
- Chloramphenicol (E)

## 2-Allergy (Hypersensitivity)

### Etiology

Drugs acting as haptens

### Examples

Penicillins

Anaphylactic shock

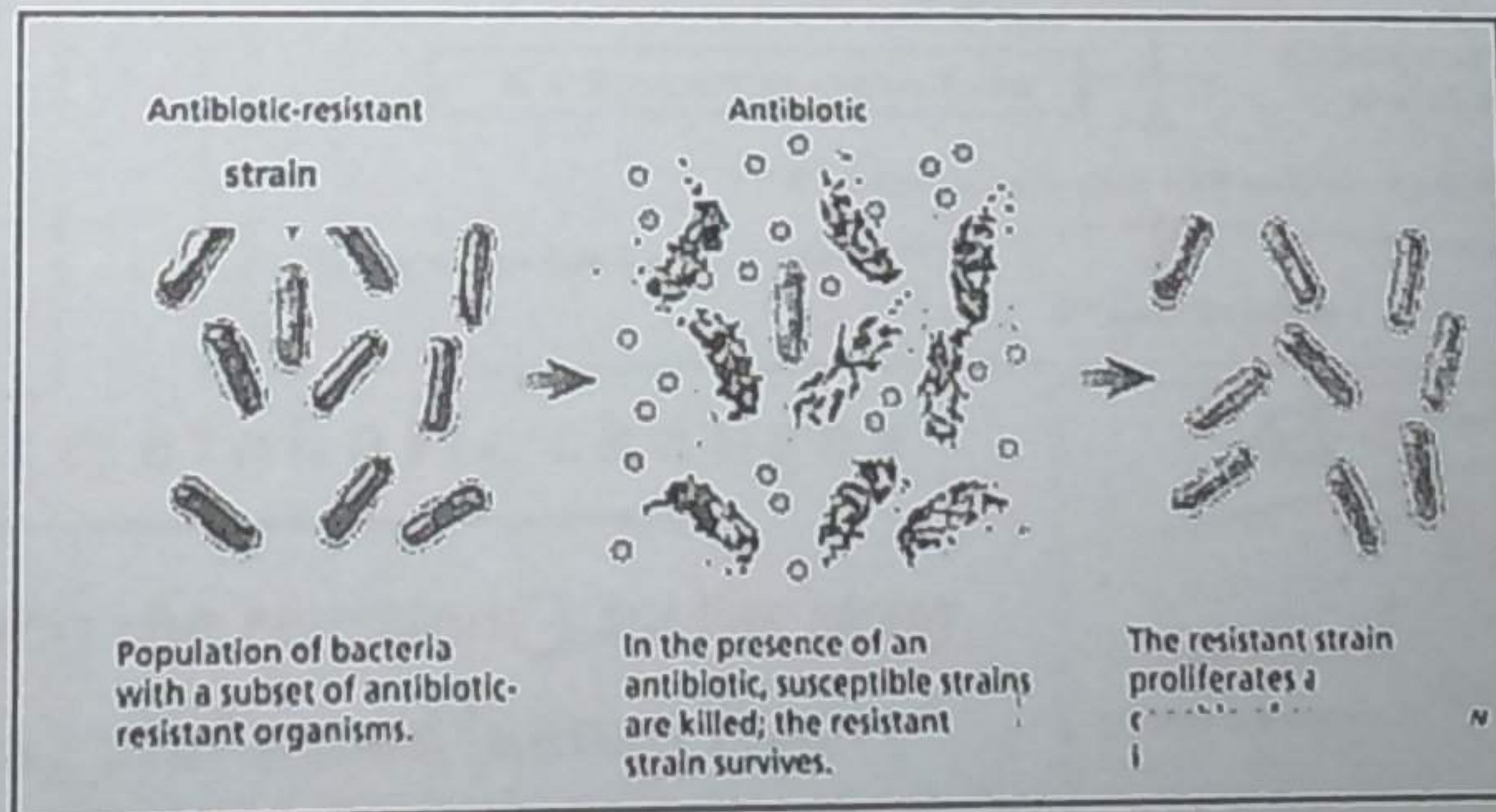
Sulfonamides (topical)

Contact dermatitis

## 3-Development of resistance

### Etiology: misuse

Low dose    Interrupted course    Wrong choice    No indication



### Consequences

Emergence & overgrowth of resistant org.

## 4-Superinfection(E)

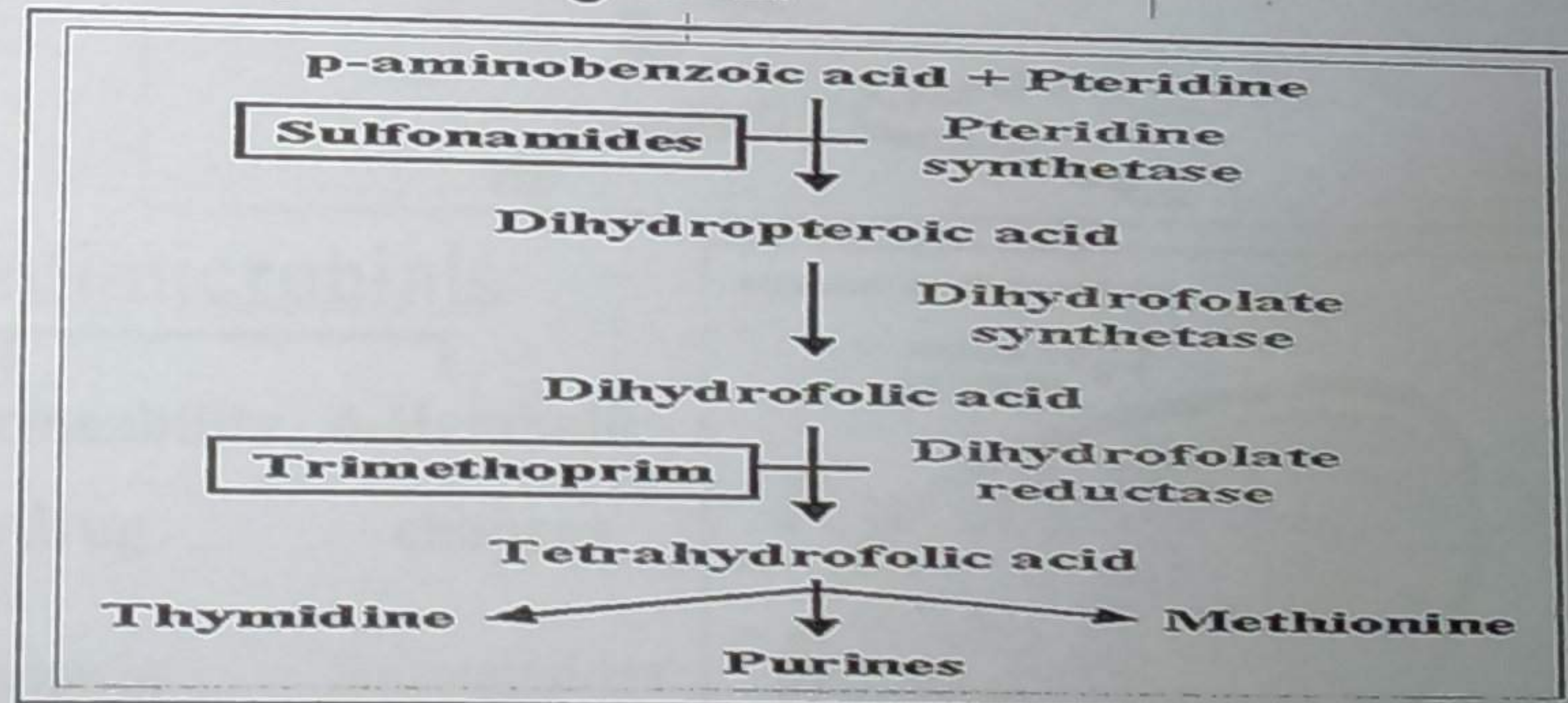
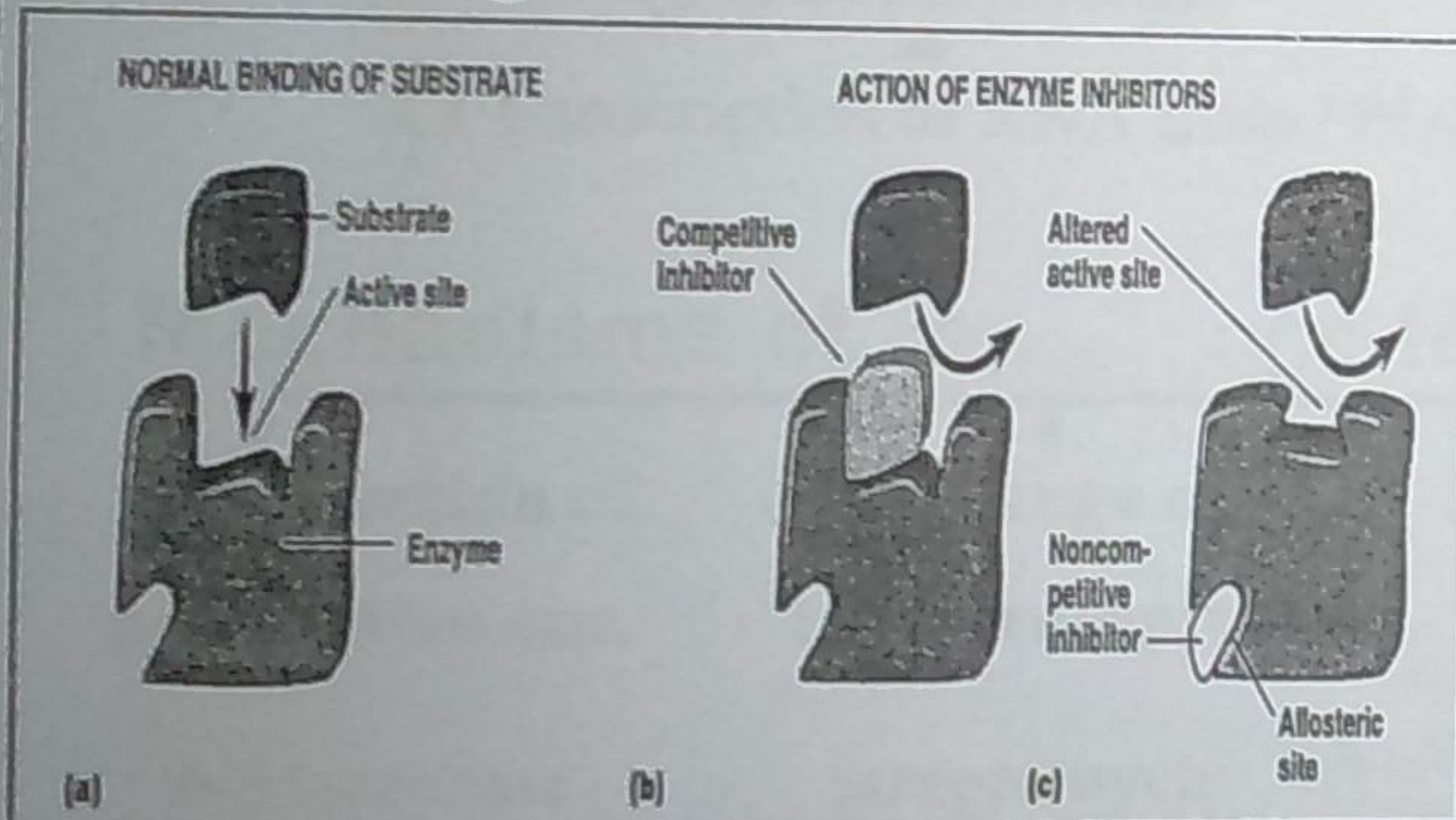
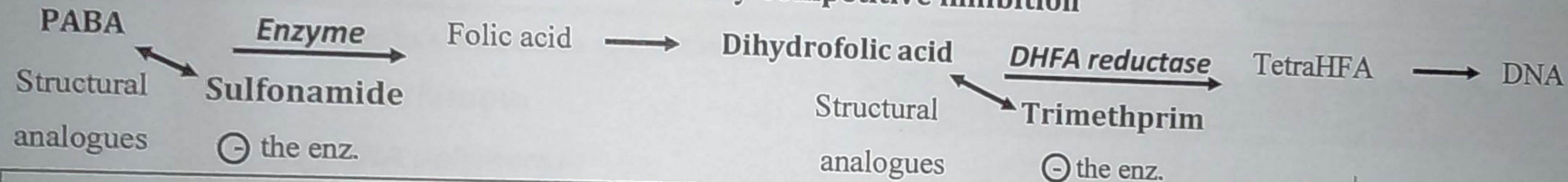
# Antibiotics inhibiting nucleic acid

Inhibition of DNA		
Indirect inhibitors	Direct inhibitors	Inhibition of RNA

## I - Inhibition of DNA

### A - Indirect inhibitors

Act by competitive inhibition



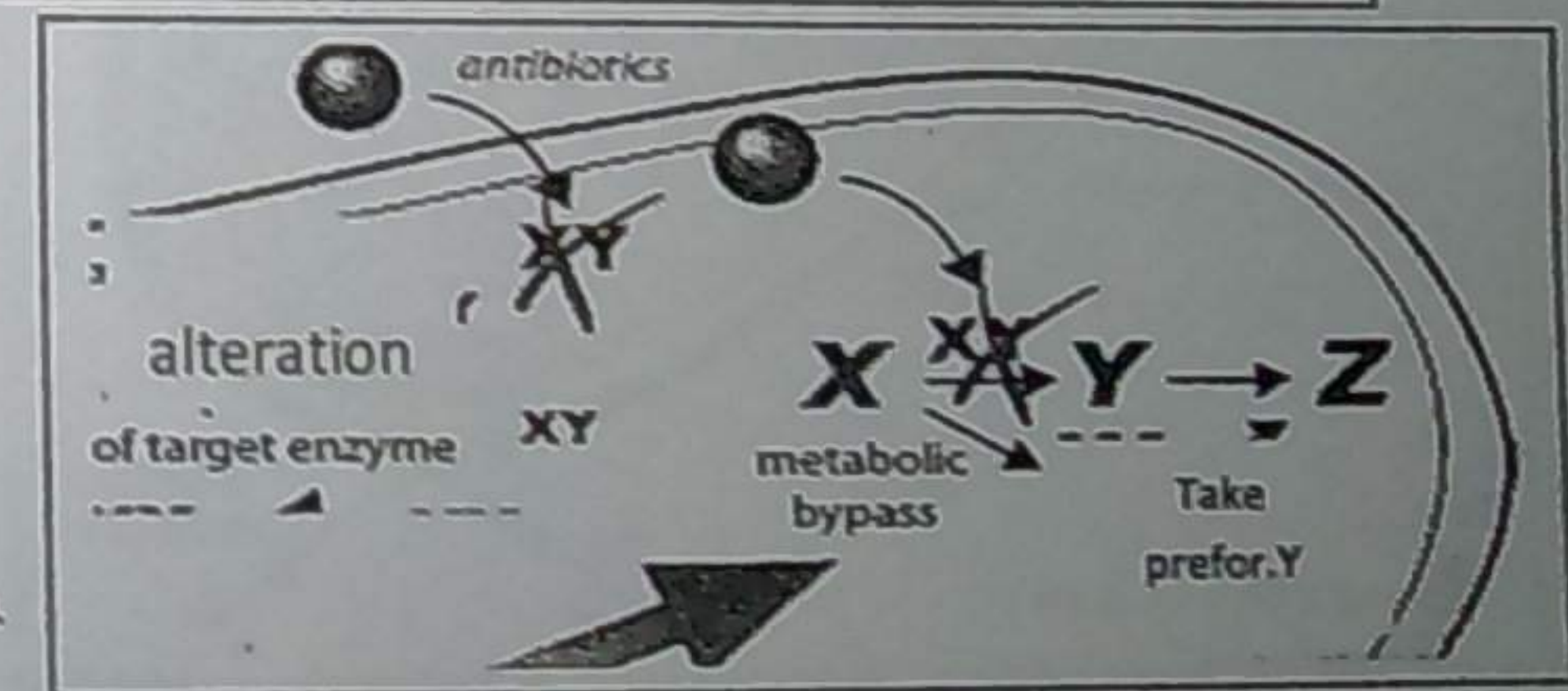
### Resistance to sulfonamides: metabolic changes

Production of altered enzyme having higher affinity for PABA

Bypassing the reaction by the drug

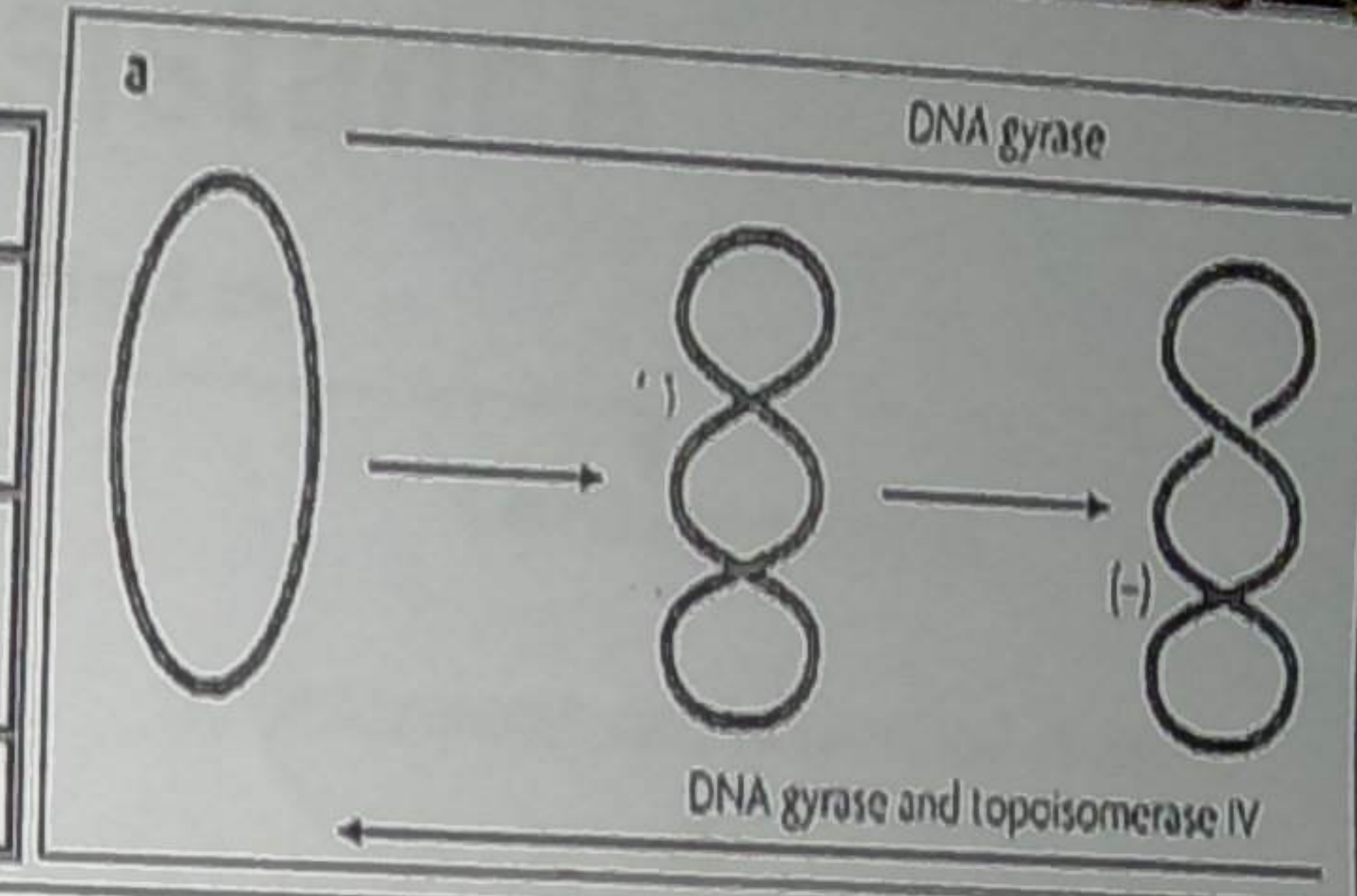
Using preformed human F.A.

9 instead of synthesizing F.A. from human PABA



## B - Direct inhibitors

	M.O.A	Examples	Uses
1-Quinolones	⊖ DNA gyrase	• Nalidixic acid • Ciprofloxacin	
2-Azoles : Nitroimidazoles	Breaks DNA	Metronidazole	i. Anaerobic bacteria ii. Protozoa
3-Novobiocin			

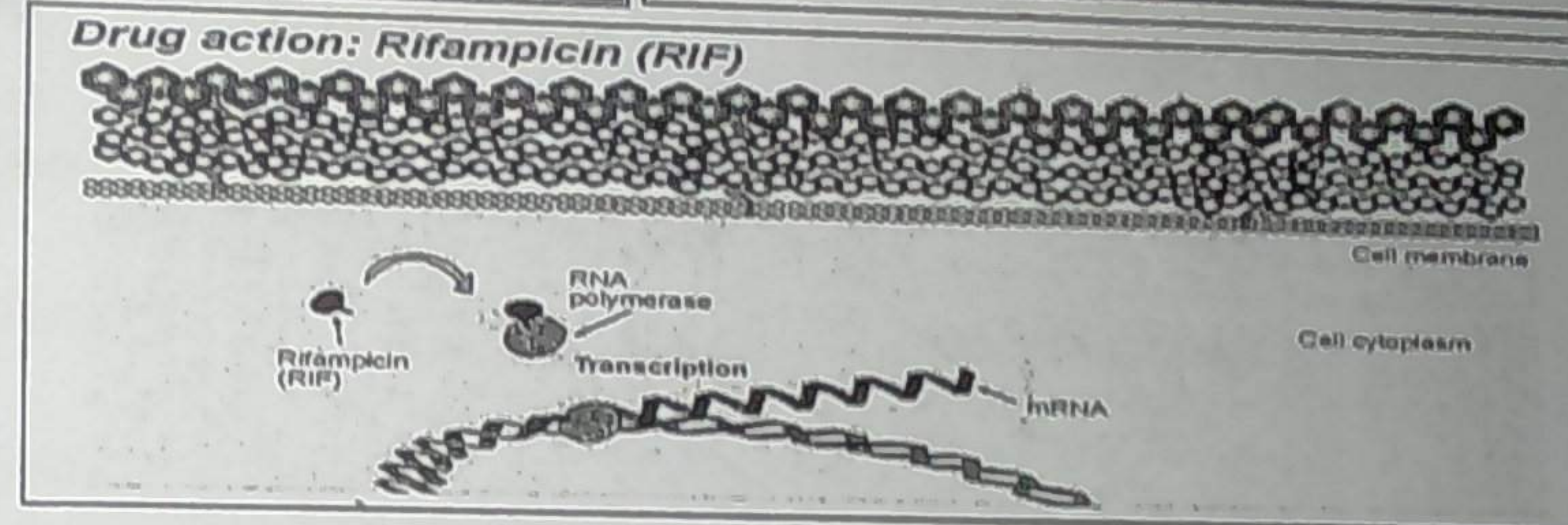


## II-Inhibition of RNA

Rifampin

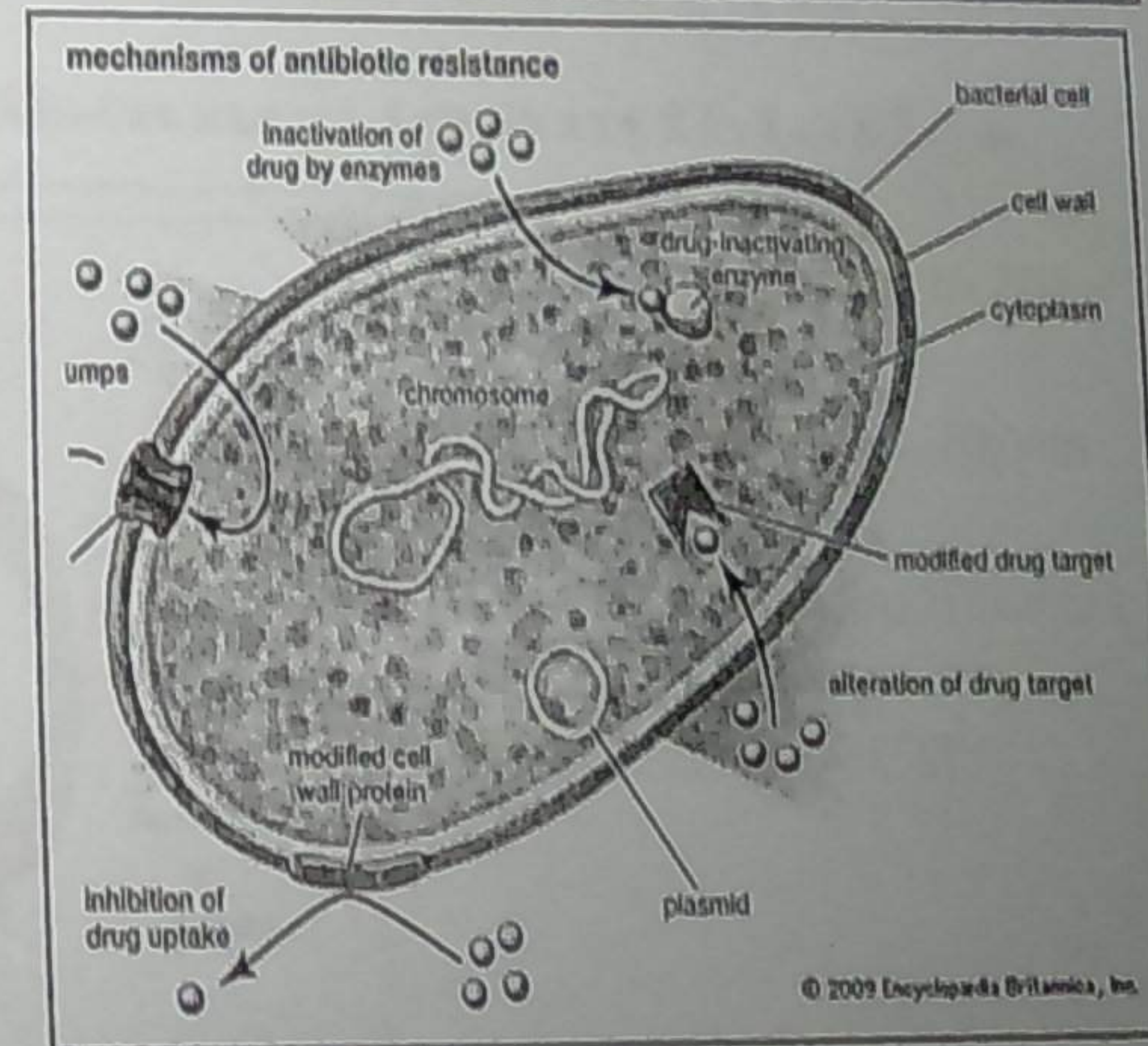
⊖ RNA polymerase

⊖ transcription of RNA from DNA



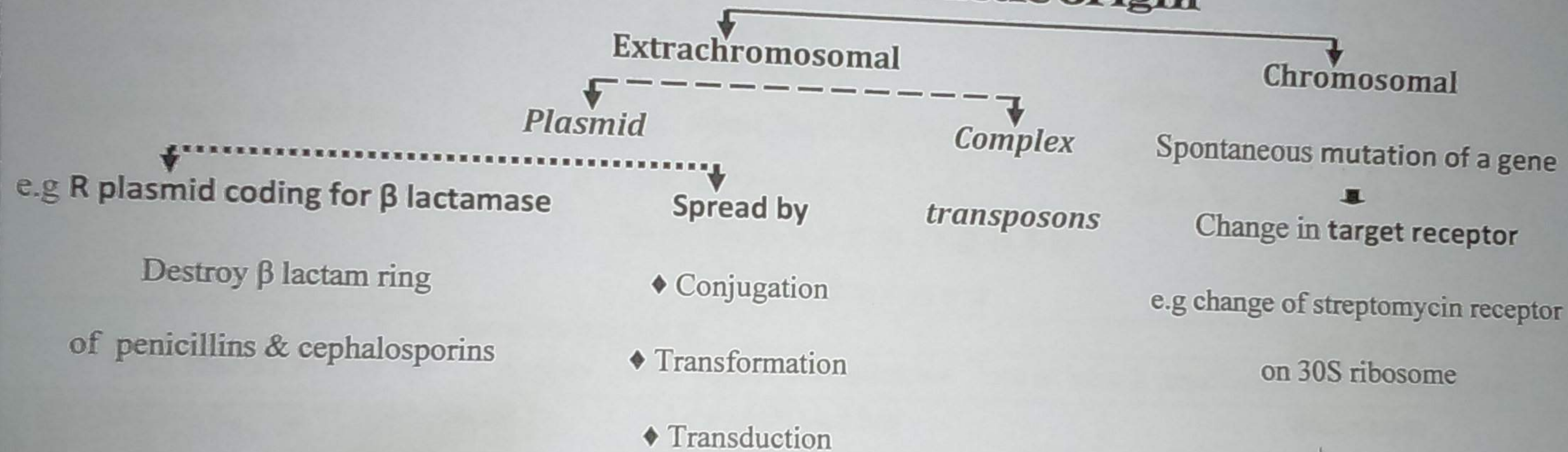
## Mechanisms of resistance to antimicrobials

1-Production of destructive enz.	2-Change of target receptor	3-↓ permeability to drug	4-Metabolic changes
♣ β lactamase & ESBLs (M)	Streptomycin (M)	Amikacin & Tetracycline (M)	Sulfonamides (M)
♣ Acetyl Transferase (M)			

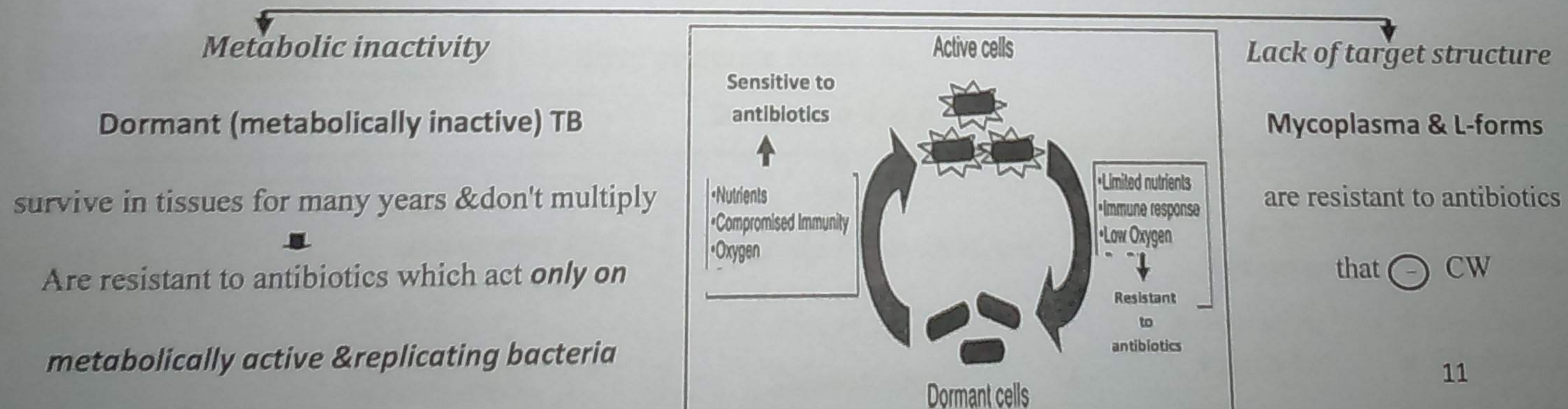


# Origin of antibiotic resistance

## A - Genetic origin

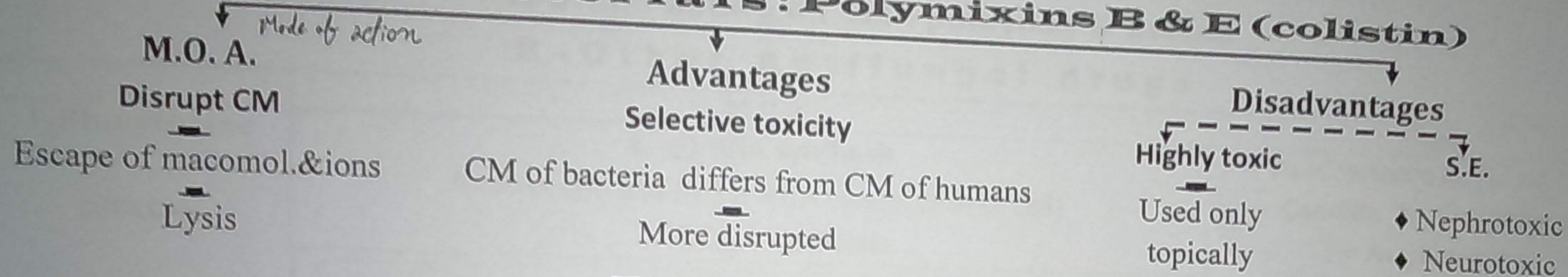


## B - Non genetic (phenotypic) origin of resistance to antibiotics



# Antibiotics interfering with CM functions

## A - Antibacterials: Polymixins B & E (colistin)

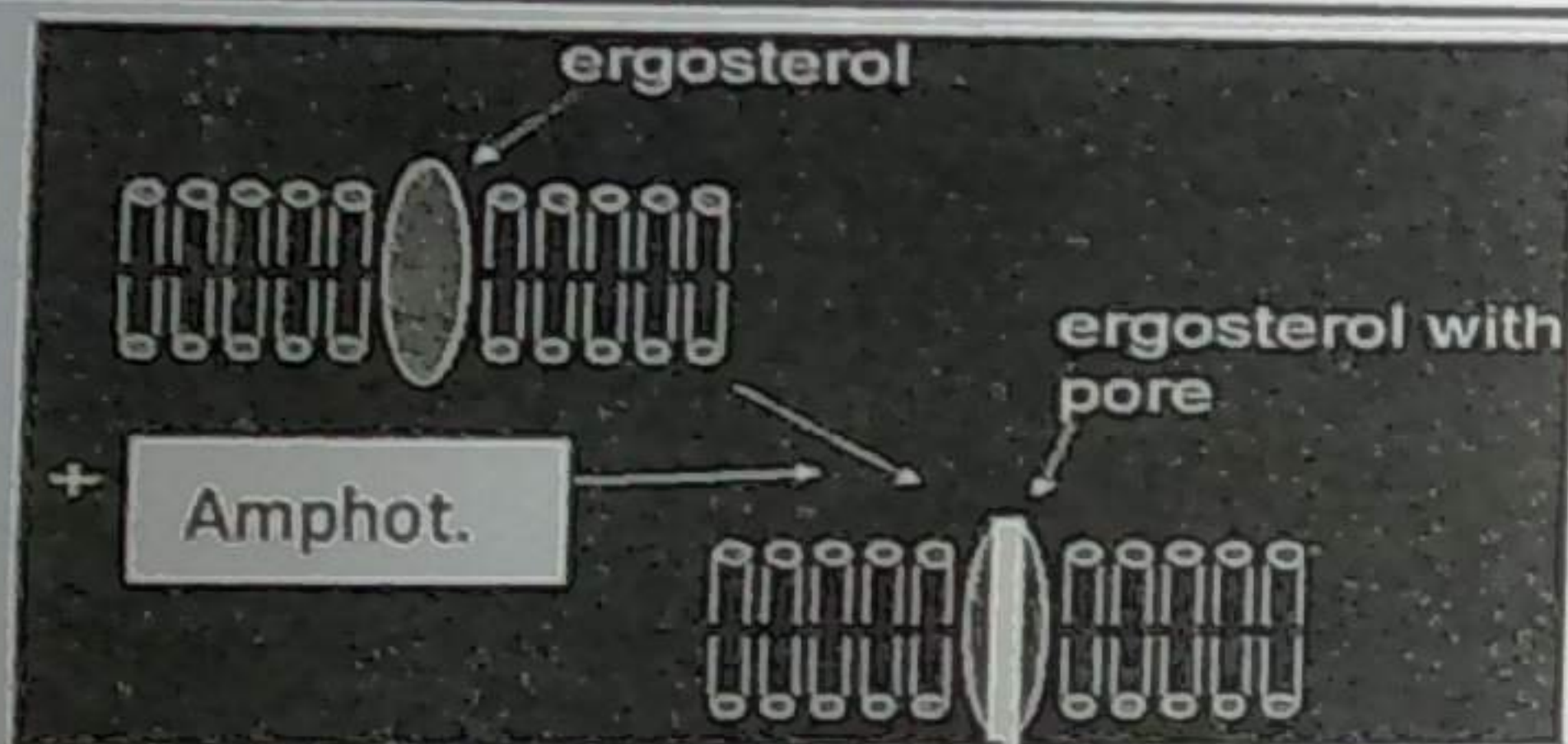


## B - Antifungals

### 1 - Polyenes

#### Amphotericin B

Bind sterols in CM → ↑ fluidity → pore formation → loss of ions & small mol. → Lysis (cidal)



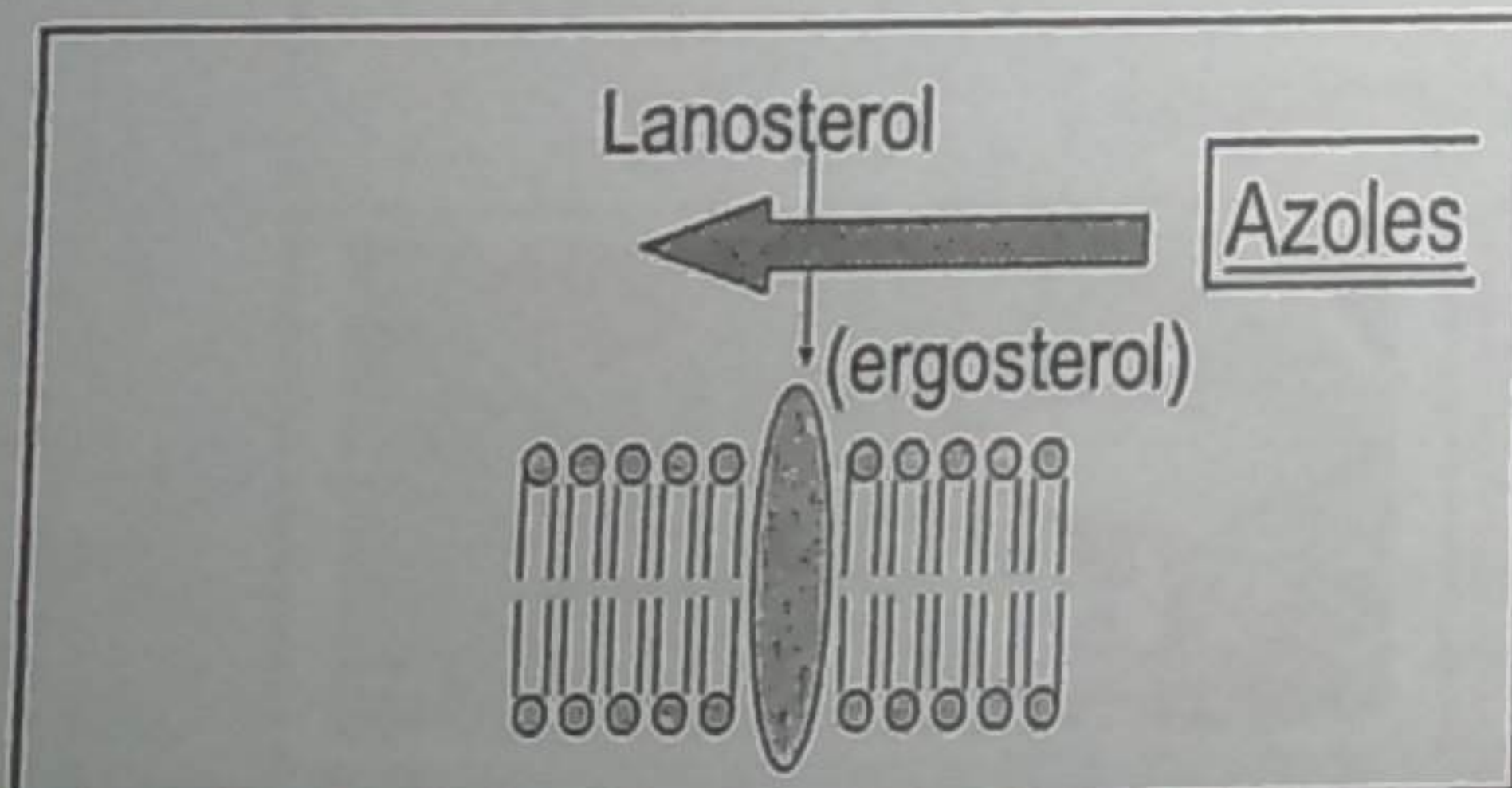
Less toxic as it has  
**greater affinity for ergosterol** (in fungi)  
 than cholesterol (in humans)  
 Ttt of **systemic** fungal inf.

#### Nystatin

**More toxic**  
 Ttt of **local** fungal skin & MM inf.

### 2 - Azoles

**M.O.A.**  
 ⊖ synthesis  
 of ergosterol



#### Uses

**Broad spectrum antifungal**

Ttt of Candida &  
 Dermatophytes

12

#### Examples

##### Systemic

- ♦ Imidazole
- ♦ Triazole

##### Topical (toxic)

- ♣ Miconazole
- ♣ Clotrimazole

# Antifungal drugs

A-Drugs acting on CM : polyenes & azoles

B-Other Antifungal drugs

1-Flucytosine

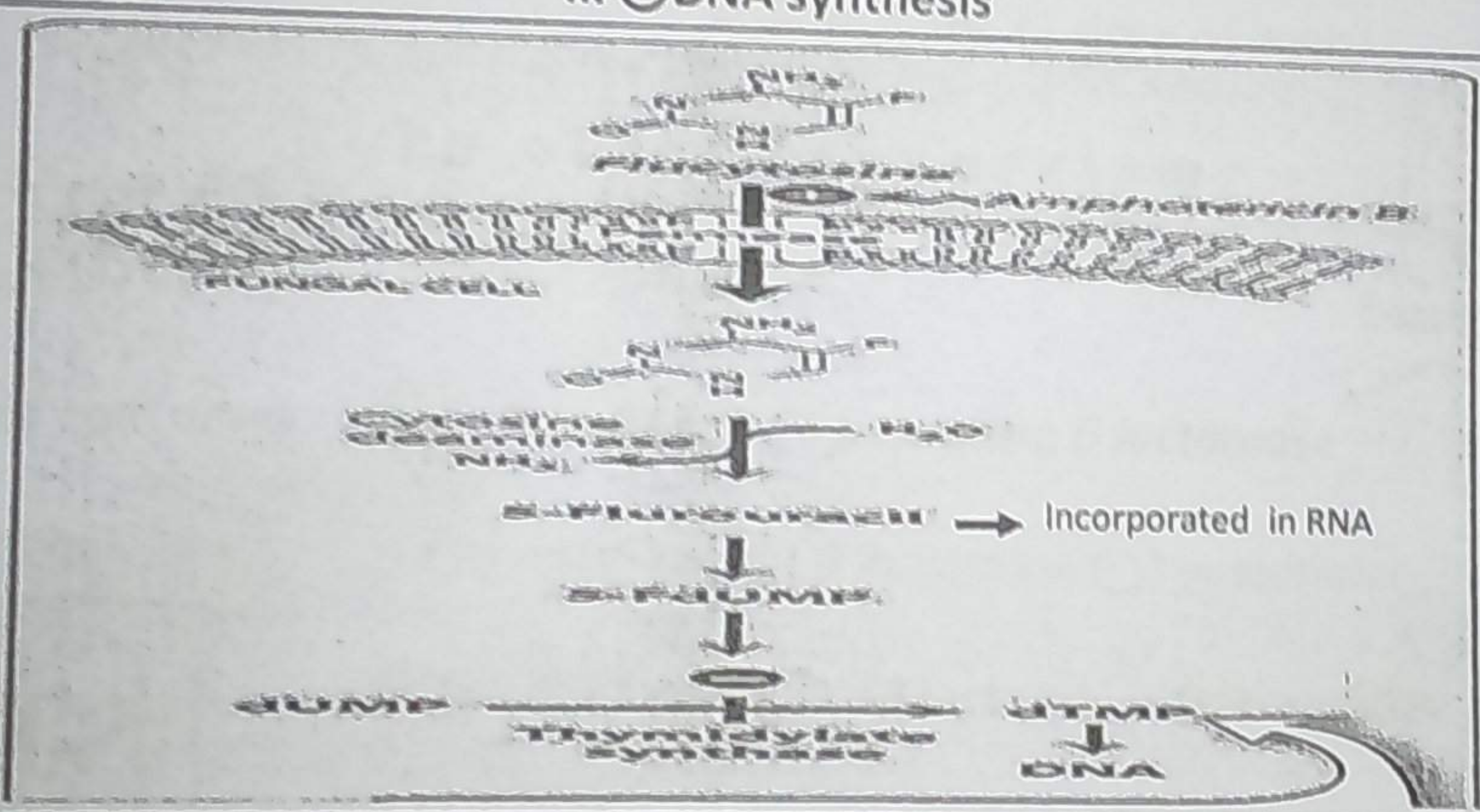
(Static)

M.O.A

i. ⊖ RNA synthesis

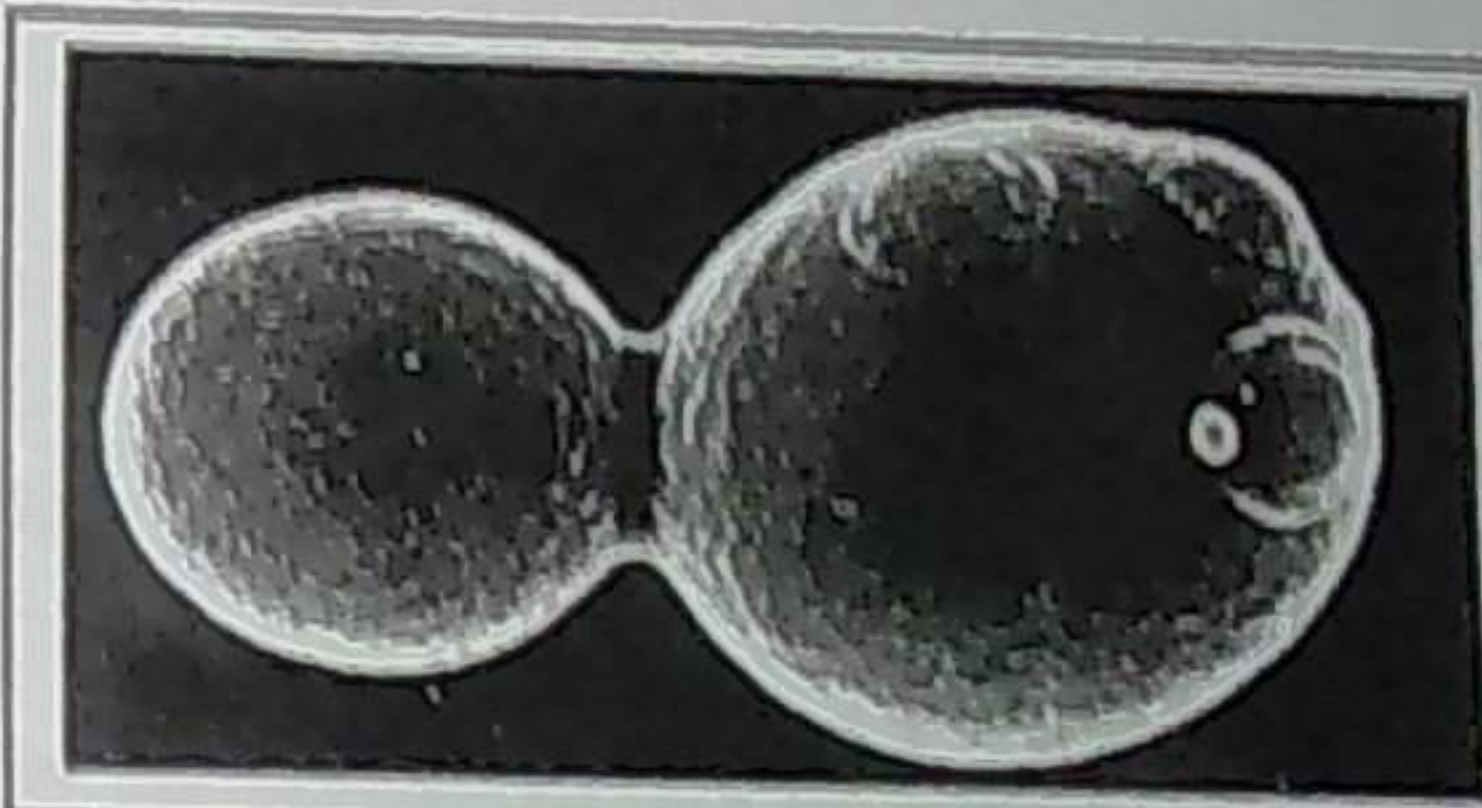
Incorporated into fungal RNA (analogue to Uracil)

ii. ⊖ DNA synthesis



Uses

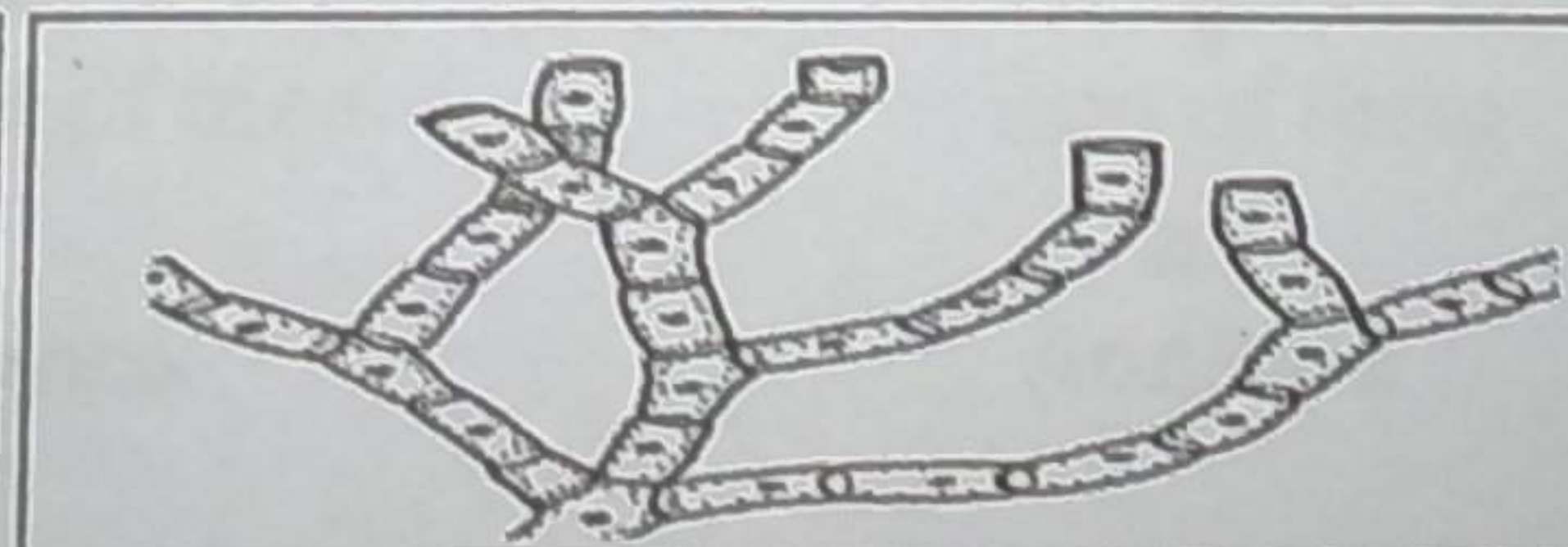
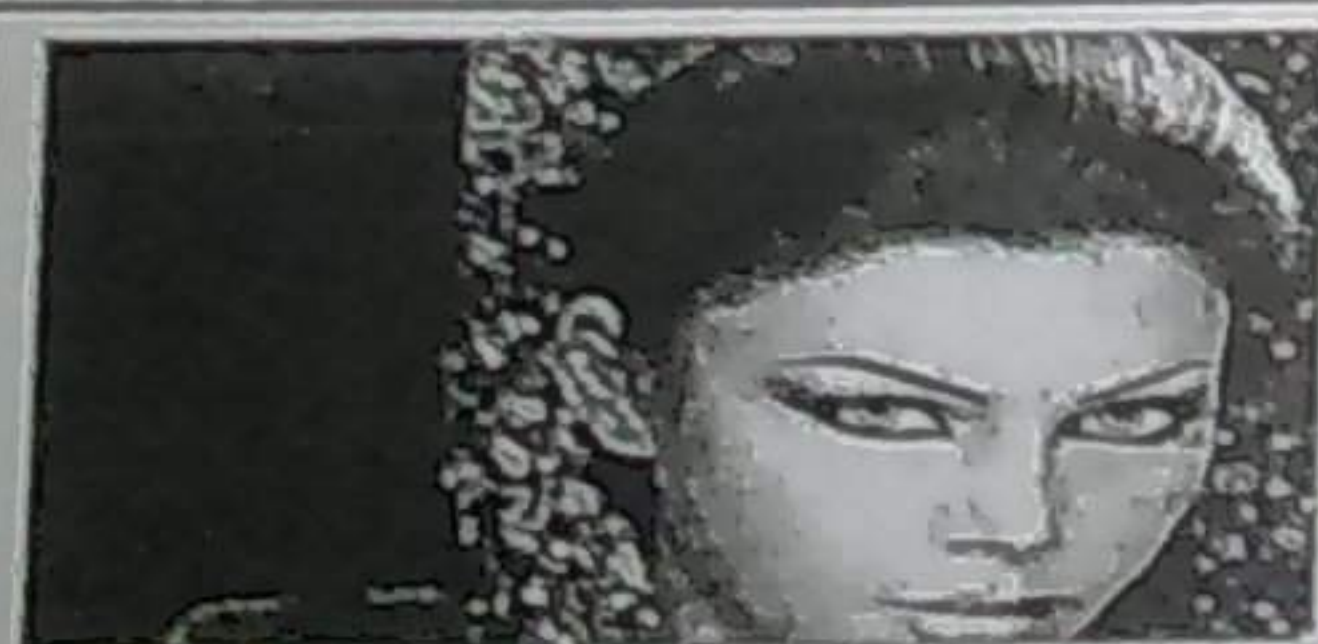
Ttt of systemic fungal inf. by  
Candida & Cryptococcus  
( + Amphotericin )



2-Griseofulvin

⊖ hyphal growth

( concentrated in keratinized tissues)



Ttt of dermatophytes inf.

( in skin , hair & nails)

# Antibiotic combinations

## A-Indications & applications

- 1-Severe undiagnosed infections  
*Septicemia*
- 2-Mixed infection
- 3-Prolonged course of ttt e.g ttt of TB
  - Prevent resistance
  - ↓ toxicity (↓ dose of each)
- 4-Complete eradication of org.  
Avoid complications

## 5-To obtain synergism

### Definition

The combined effect of both drugs

is > sum of them

**Synergy**

$$1+1 = 3$$

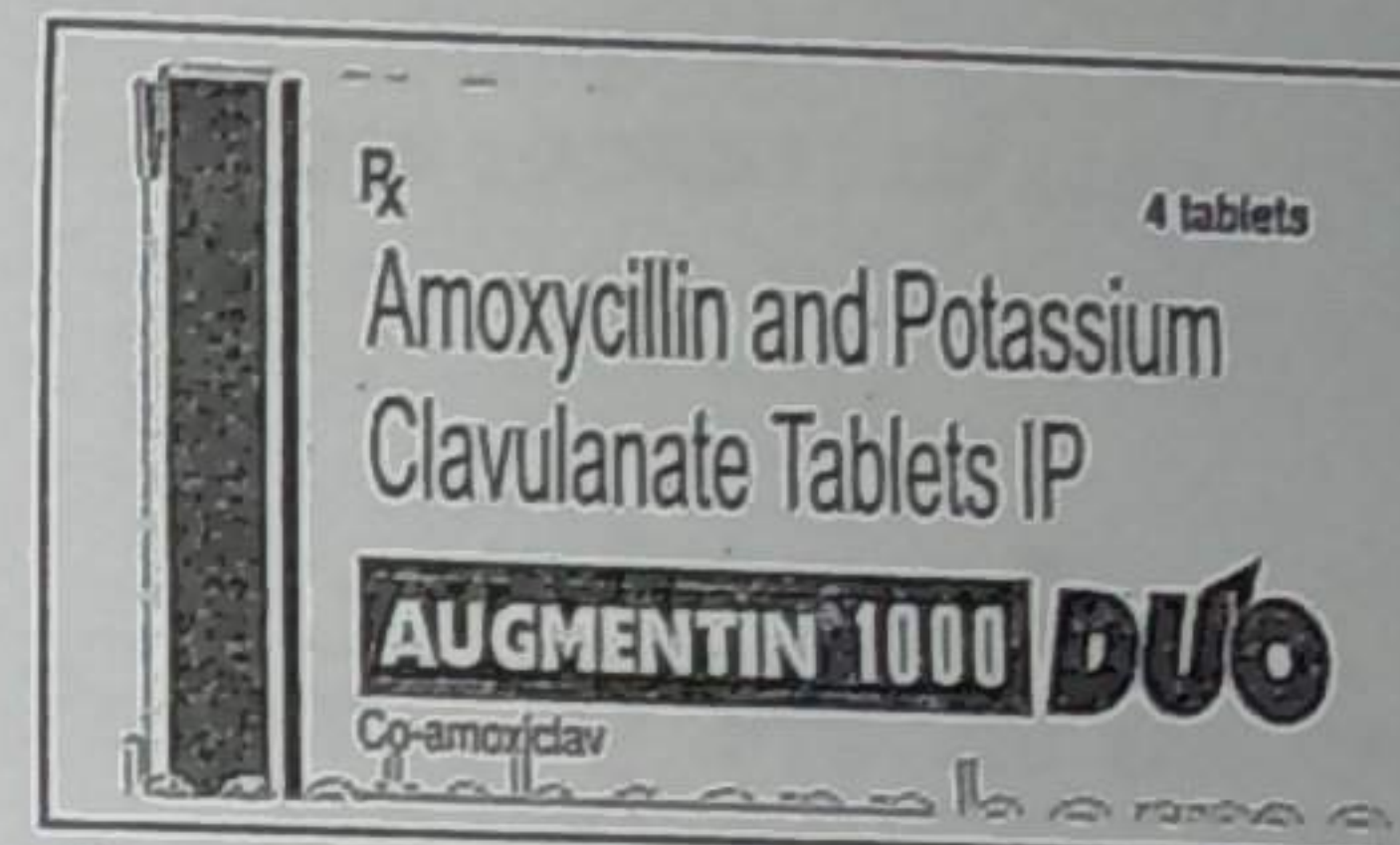
### Examples

Ttt of bacteria producing  $\beta$  lactamase

Clavulonic acid (  $\beta$  lactamase  $\ominus$  ) potentiates  
action of amoxicillin (  $\beta$  lactam ) → Coamoxiclav

Ttt of Streptococcal endocarditis

$\beta$  lactam drug potentiates  
action of aminoglycosides



## B-Effects

The combined effect of both drugs may be

< most potent of them

Antagonism

= most potent of them

Indifference

= sum of them

Addition

> sum of them

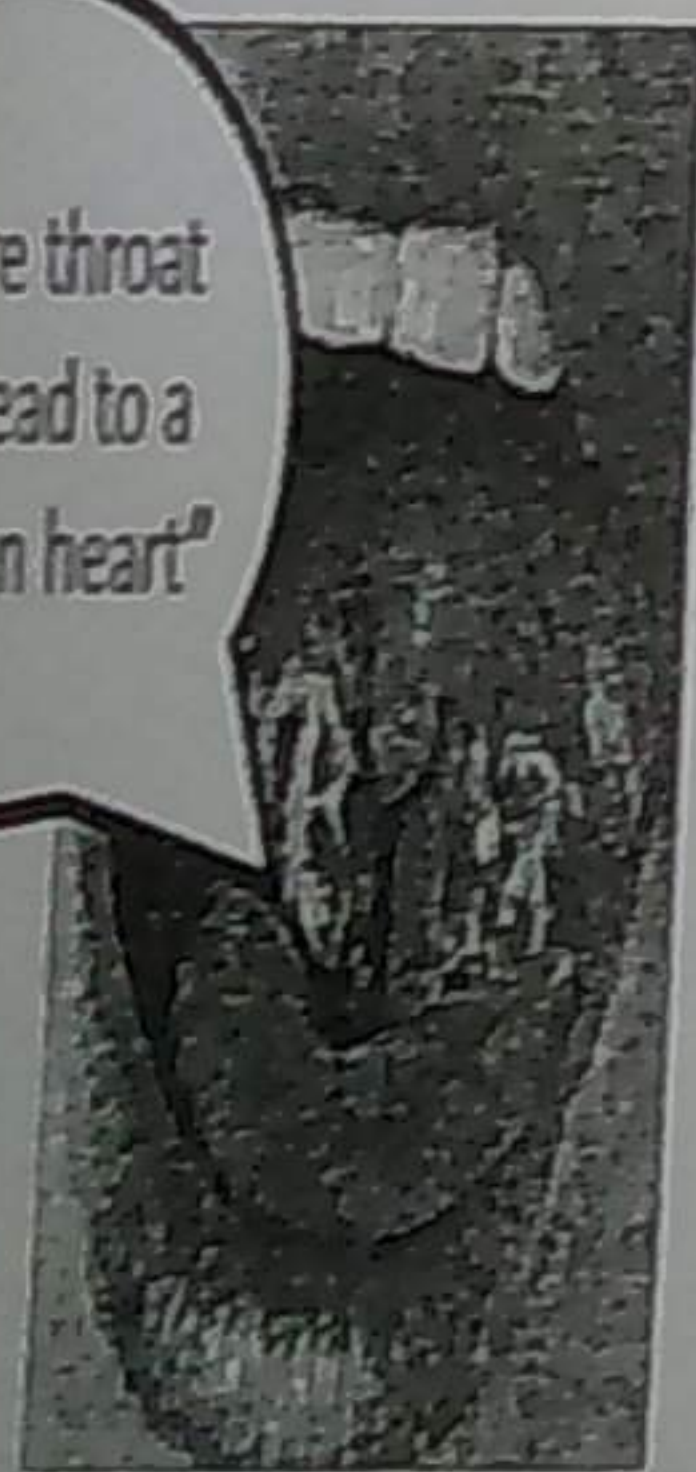
Synergism

# Prophylactic use of antimicrobials

## I-Medical prophylaxis

Rheumatic fever	Subacute bacterial endocarditis	Meningitis	Cholera
Penicillin G every 4 ws  to pts with rheumatic fever  ↓	Single dose of amoxicillin  before dental operations  or tonsillectomy  ↓	Rifampicin  for close contacts of case  ↓	Tetracycline  for close contacts of case  ↓
Prevent <b>recurrence</b>  of throat inf. by <i>Streptococcus pyogenes</i>	Prevent SABE (by <i>Strep. viridans</i> )  in pts with <b>congenital or</b> <b>rheumatic heart ds</b>	Prevent  <i>meningococcal</i> <i>meningitis</i>	Prevent <b>cholera</b>

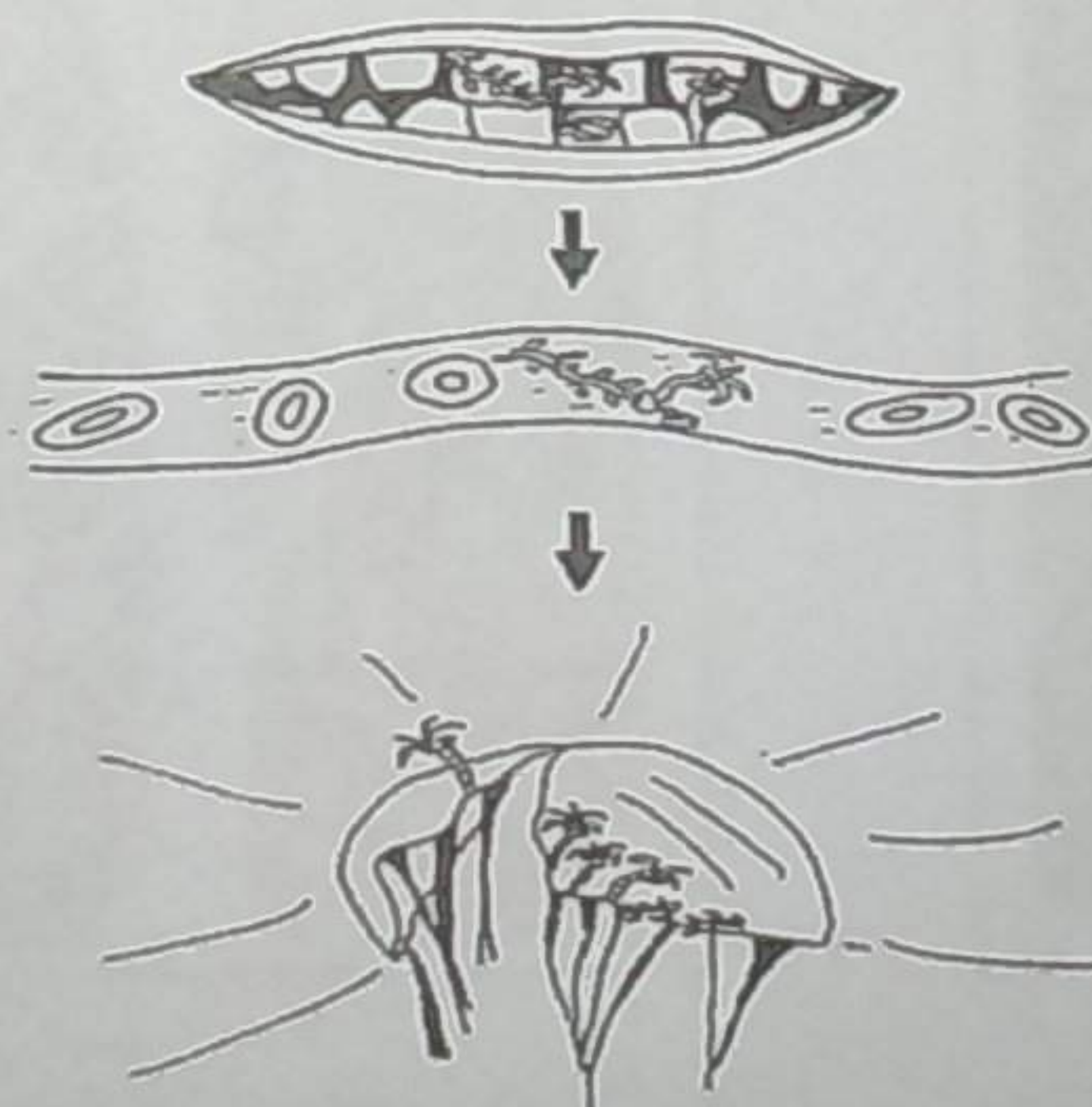
"A sore throat  
can lead to a  
broken heart"



Strep Throat

Rheumatic  
Fever

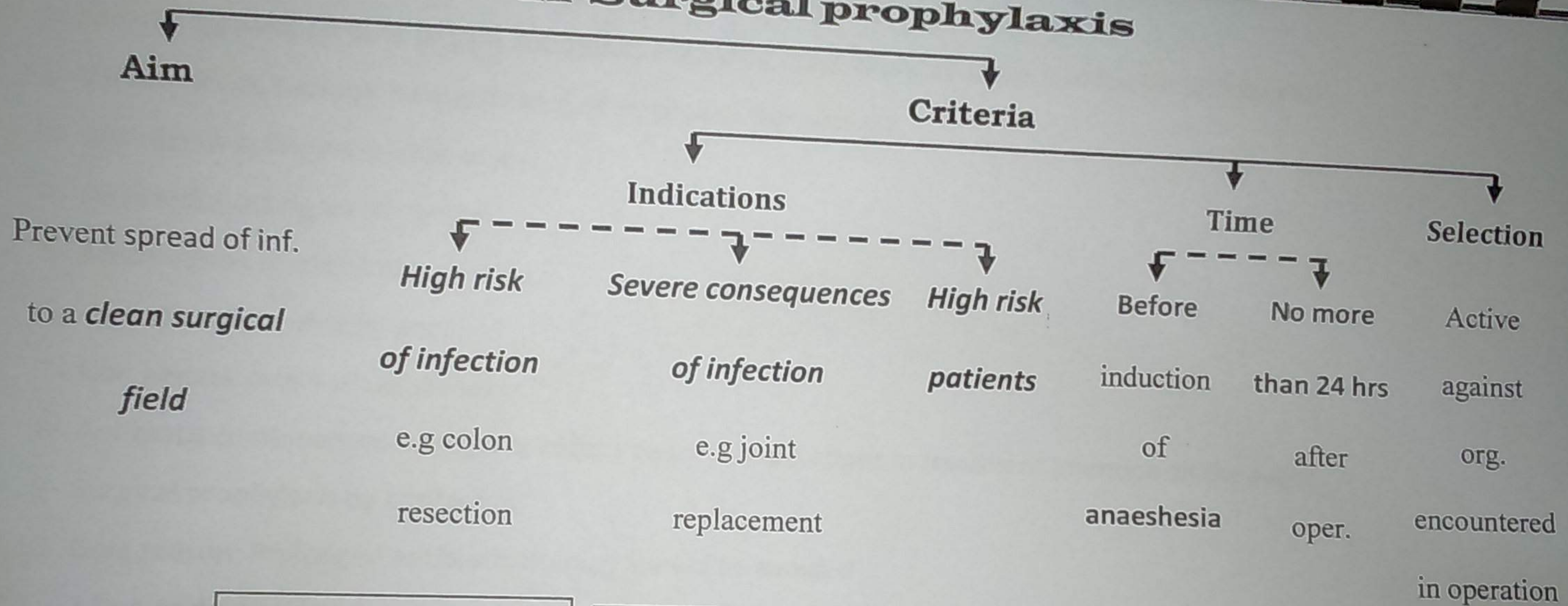
Rheumatic  
Heart Disease



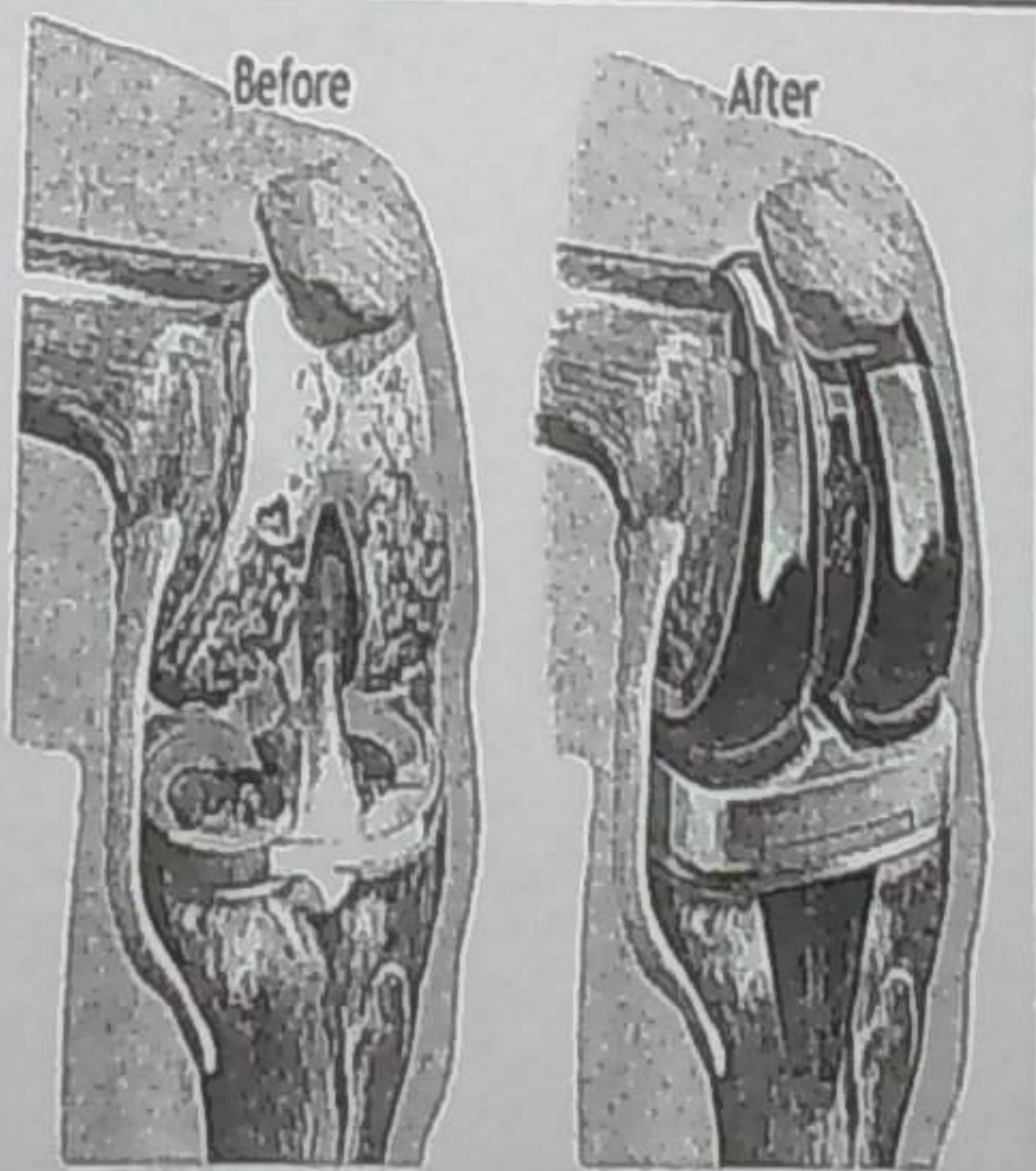
(VIRIDANS) FOLIAGE GROWING  
ON MITRAL VALVE



## II-Surgical prophylaxis



Removal of affected bowel



### Essay questions

- 1- Give an account on Beta lactam antibiotics regarding mechanism of action and bacterial resistance.
- 2- Define MRSA, mention its mechanism of resistance & treatment.
- 3- Antibiotics acting on nucleic acids.
- 4- Antibiotics acting on ribosomes.
- 5- Mechanisms of antibiotic resistance.
- 6- Genetic origin of resistance.
- 7- Non genetic origin of resistance.
- 8- Antibiotic combinations as regards indications and applications in treatment (mention all the page).
- 9- Surgical prophylaxis by antibiotics.
- 10- **Give reason:** Prolonged antibiotic therapy should be avoided  
( Due to drug toxicity , resistance & superinfection).
- 11- **Give an account on :**
  - a. Synergism.
  - b. Superinfection.